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UNIVERSITY OF ALBERTA

Startle response in the Colorado potato beetle, *Leptinotarsa decemlineata* (Say)(Coleoptera: Chrysomelidae)

BY

© **Susanna Acheampong**

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Master of Science.

DEPARTMENT OF ENTOMOLOGY

Edmonton, Alberta

SPRING 1994

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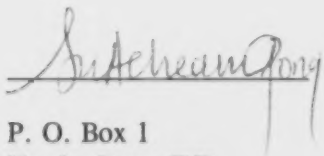

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled Startle response in the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) submitted by Susanna Acheampong in partial fulfillment of the requirements for the degree of Master of Science.

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Dr. B. S. Heming

Dr. S. D. Pollard

DATE : April 12, 1994

DEDICATION

To my husband, J. K. A. Bamfo and my parents, Nana Adarkwa Boadi Yiadom II and Mrs Elizabeth Acheampong.

ABSRACT

The startle response in the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) was induced by dropping bell sinkers with weights varying from 14-134g on a support on which beetles rested and by controlled stimuli from a large acoustic speaker. Results from preliminary experiments showed that recovery from the immobile state was either preceded by leg or antennal movement. The time taken for beetles to come out of the immobile state (i.e. recovery time) was not dependent on age or sex, though pre-startle behavior did affect recovery time.

The stimulus caused by a falling sinker was in the form of sine waves with a frequency of 110Hz for all sinkers used. A more controllable device was thus designed to startle beetles. The set-up involved the use of a loudspeaker, frequency generator, frequency counter, timer and amplifier. 30Hz was found to be the most effective frequency for startling beetles. Recovery time was not affected by stimulus duration, but was dependent upon displacement and satiation level of beetles. Starved beetles took a shorter time to recover than fed beetles. Repeated presentations led to a trend of sensitization followed by habituation.

Eyes and antennae are not involved in the detection of vibration. A chordotonal organ is present in the trochantero-femoral joint of all three legs and a novel mechanosensory organ only in the forelegs. Results of amputation experiments showed that the novel organs were not the only organs involved in the detection of the startle stimuli.

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CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

Thanatosis (death feigning) is practised by such diverse organisms as insects, crustaceans, birds, reptiles and mammals (Frost, 1942). In mammals, thanatosis is well developed in the American opossum, *Didelphis* spp. and the African ground squirrel (Ewer, 1966). In the opossum, *D. marsupialis*, tactile stimulation or grabbing by a predator is necessary to induce thanatosis and the duration of feigning increases with daily stimulation up to 60 days (Franq, 1969).

In insects, thanatosis has been found in Coleoptera, Orthoptera, Phasmida, Hemiptera, Odonata, Lepidoptera and Plecoptera. In Coleoptera, thanatosis is induced by disturbing a beetle or when it loses contact with the substratum, due to activity of a predator (Wigglesworth, 1964). Thanatosis is found in members of various families of Coleoptera. It is common in darkling beetles (Tenebrionidae). In the flour beetle, *Tribolium castaneum*, death feigning behavior was induced by dropping it onto a small china plate and tapping it lightly on the anterior end with a camel-hair brush (Prohammer and Wade, 1981). These authors found that recovery from the immobile state was often preceded by movement of the antennae, and that the duration of death feints was strongly negatively correlated with the tendency to perform feints i.e. beetles which required a greater number of taps to induce death feints remained in the quiescent state for shorter periods.

The brentid, *Arrhenodes minutus*, like most Curculionoidea, feigns death when disturbed (Sanborne, 1983). When approached cautiously and tapped with a pencil point while it is feeding on asparagus foliage, the asparagus beetle, *Crioceris asparagi* exhibits thanatosis (Capinera, 1975). Thanatosis has also been observed in the following Coleoptera: *Lucon murinus*, *Cidonopus aeruginosus*, *Cassida viridis* and *Strangalia quadrifasciata* (Reitze and Nentwig, 1991).

In the phasmid, *Carausius morosus*, death feigning is dependent upon illumination. Thanatosis is exhibited when a cage of active insects in the dark is

suddenly illuminated. It can also be induced in this insect by turning it on its back. Stimulating the tip of the abdomen and the pretarsal segments of the legs however, brings about a rapid recovery from thanatosis (Godden, 1972).

Holmes (1906) showed that death feigning is developed to an unusual degree in the water scorpion, *Ranatra* (Hemiptera). Picking up a bug in the fingers, gently stroking and dropping it on a table induces thanatosis. *Ranatra* will resume feints when stroked or touched or even when a puff of air is blown on it at the first sign of recovery from a feint. In very susceptible individuals, death feints may be induced by touching the thorax, legs or abdomen. Duration of feints decreased with successive trials. Exposure to higher temperatures led to a reduction in duration of feints while lower temperatures had the opposite effect. In an experiment with two groups of eight specimens each, with one group kept at 30°C and the other kept at temperatures from 10-14°C, the mean duration of feints for the 30°C group was 43 min compared to that of 137 min. for the 10-14°C group. Duration of feints was also modulated by light. In an experiment where a group of specimens was exposed to dim light and another group to bright light, the mean duration of feints for the dim light group was 116 min and for the bright light group was 75.8 min. Removal of the supraesophageal ganglion caused a marked decrease in the duration of feints (Holmes, 1906).

Holmes (1906) also found that a group of swimming *Ranatra*, when approached, will cease all movement and lie with their legs outstretched. This behavior, called 'deceptive quietness' by Holmes, is exhibited by this insect in the presence of natural enemies.

In Odonata, members of the endemic Hawaiian genus *Megalagrion* exhibit thanatosis. This behavior was found in four species which differed greatly in size and color (Moore, 1983): *Megalagrion blackburni* (large and red), *Megalagrion hawaiie* (small and red), *Megalagrion nigrohamatum* (a black and yellow species) and *Megalagrion pacificum* (small and black). But there was no relation between thanatosis

and size or color. Death feigning did not always occur with repeated presentations to the same insect. When a female *M. blackburni* was picked up 13 times it showed the following sequence of responses: immobilization on the first 6 occasions, none on the next 4, immobilization on the next, none on the next and immobilization on the last. Duration of feints was reduced from 7 min. in the first to 0.5 min in the last. However, there was no such regular trend for the other species. In *M. blackburni* and *M. hawaiiense*, recovered from thanatosis after gently blowing on them. For *Megalagrion* species, Moore (1983) suggested that thanatosis is a mechanism to escape from the large odonate predator, *Anax strenuus*.

In *Caligo illioneus*, a neotropical butterfly, thanatosis was induced by grasping the base of the folded forewings and removing the insect from its resting substrate. In the thanatonic condition, there was complete absence of wing or leg movement, with the legs tucked against the body as in flight (Dudley, 1990).

Nymphs of *Pteronarcys dorsata* (Plecoptera) use thanatosis and "freezing" to resist attack from fish predators. Thanatosis was exhibited when the nymphs were lifted off the substrate by the predator and freezing ensued when a trout attacked but was unsuccessful in loosening the nymph's grip on the substrate. When a trout touched the body or cerci of nymphs, they frequently exhibited thanatosis and when the antennae or legs were touched they exhibited freezing. Freezing in nymphs was characterized by clinging to the substrate while thanatosis was associated with curling up of the nymph. Duration of thanatosis increased significantly with successive attacks when *P. dorsata* was attacked repeatedly by trout (mean of 2.03 ± 0.51 min after first attack compared to 22.4 ± 5.89 min after second attack) (Moore and Williams, 1990).

The freezing (stopping and clinging) reaction has been observed in the honey bee, *Apis mellifera*, where it was induced by sound frequencies between 500 and 1000 Hz (Little, 1962). Little (1962) conducted experiments to determine whether the stopping-reaction was due to the detection of air-borne sound or of vibration and

concluded that substrate vibration was the effective stimulus. By removal of the flagella of the antennae, as well as pairs of legs, he concluded that the antennae did not play any role and that all three pairs of legs were involved in vibration detection (Little, 1962).

Adaptive Significance of Thanatosis

Thanatosis may be of survival value to a prey animal because it causes a predator to relax its attention, allowing for active escape of prey. Also, since predators are often not interested in dead prey, motionless insects may be ignored (Edmunds, 1974).

Reitze and Nentwig (1991) investigating the feeding ecology of six mantid species, observed that the beetles, *Lucon murinus* and *Cidonopus aeruginosus* were not attacked for hours when motionless but as soon as they moved they were attacked. *Cassida viridis* did not move at all in the feeding trials and thus were not attacked and eaten. The small and active cerambycid, *Strangalia melanura* was eaten more often compared to the large and inactive species *S. quadrifasciata* which was only rarely eaten. In phasmids, these authors found that thanatosis has evolved to such an extent that they moved very little, and when seized by mantids they did not struggle to get away but remained motionless, which resulted in their being set free without any damage.

Mantids possess both mobile and immobile tibial thorns on their forelegs (Copeland and Carlson, 1977). These are mechanoreceptors and during prothoracic tibial flexion, they inform the mantid about movement of prey in the catching leg. If the tibial thorns do not signal a struggling prey, the forelegs open in preparation for the next strike. Thus in phasmids, the wrong message of "empty forelegs" is sent (Reitze and Nentwig, 1991). Though diplopodes have powerful defense glands, they were often attacked after their very first movement. Reitze and Nentwig 1990 thus

concluded that thanatosis was a better defence against mantids than was chemical defence.

The stopping reaction is known to be of practical value in apiculture. It can be induced by use of inexpensive vibrators attached to hives (Frings and Little, 1957), allowing hive inspection while the stopping-reaction was in progress (Little, 1962). This method of inspecting hives was more effective than smoke, leading to no stings and fewer bees leaving the comb. An additional advantage was that bees did not have to rid the hive of smoke.

Movement and Vibration Detection in Insects

Chordotonal Organs

Chordotonal organs are a group of mechanoreceptor cells attached to an immobile part of the cuticle and connected by a ligament to another part of the cuticle that is mobile relative to the first. The receptors measure the deformation caused by the elastic stretch or contraction of the ligament (Frantsevich and Shumakova, 1985). There are four types of chordotonal organs: simple chordotonal organs which detect position of the antennae, wings and legs; subgenual organs concerned with the detection of vibrations of the substrate, tympanal organs, and Johnson's organs which are located in the pedicel of the antenna of some insects and detect movement of the antennae under the influence of gravity or wind in flight. Johnson's organ can also serve as a ballistic organ when the animal is suddenly displaced, and as a modified hearing organs (Bullock and Horridge, 1965).

Subgenual Organ

These are specialized chordotonal organs located on the proximal end of the tibia and suspended between the trachea and the hypodermis just below the femur-tibia

joint (Bullock and Horridge, 1965). Subgenual organs vary in shape depending upon the insect order and it has been described as cone-, fan- or sail-shaped (Friedman, 1972). They are fan-shaped in the orthopterans (Schwabe, 1908) and dictyopterans (Friedrich, 1929; Debaisieux, 1938), and club- or cup-shaped in termites (Richard, 1950; Howse, 1965). In lepidopterans, subgenual organs are unattached distally and are diffuse (Debaisieux, 1935). The sensitivity of the subgenual organ depends upon its shape, with the fan-shaped variety being the most sensitive (Autrum and Schneider, 1948).

In plecopterans, subgenual organs play a role in mating, detecting vibrations of the substratum set up by drumming (Rupprecht, 1968). In *Periplaneta,* the subgenual organ is sensitive to frequencies from 1 to 5KHz with threshold amplitudes as low as 10^{-7} to 10^{-10} cm (Autrum and Schneider, 1948; Schnorbus, 1971; Friedman, 1972). Imms (1977) was of the view that some Hemiptera, Coleoptera and Diptera lack subgenual organs while Wigglesworth (1972) stated that though subgenual organs are poorly developed in Hemiptera, Coleoptera and Diptera simply do not have them. Members of these three orders detect vibration by means of tibio-tarsal chordotonal organs and hair sensilla in the tarsal joints (Autrum and Schneider, 1948).

Femoral Chordotonal Organ

Another well known chordotonal organ is the femoral chordotonal organ (FCO). In the Ortopteran, *Locusta migratoria*, the FCO is known to consist of two regions; the proximal and distal scoloparium. The distal scoloparium mediates the resistance reflex in *Locusta*, and while the role of the proximal scoloparium is not clear; it is speculated that it contributes a vibration-sensitive component. Thus the femoral chordotonal organ and subgenual organ participate the overall alarm response of the locust (Field and Pfluger, 1989).

Purpose of Thesis

The Colorado potato beetle, *Leptinotarsa decemlineata* (Say) exhibits thanatosis when picked up or when touched, the legs being either extended or withdrawn close to the body (Figure 1). When the plant on which they are moving is disturbed, beetles cease movement and remain immobile for some time before resuming normal activity (Mitchell, personal communication). This behavior is termed the 'startle response' in this thesis (Mitchell, personal communication). Though thanatosis is exhibited by the beetle, it has not been examined experimentally. In their paper on the defensive mechanisms of the beetle, Pasteels and Deroe (1977) summarized its various defense mechanisms as being: production of glandular secretions, reflex bleeding, defecation and regurgitation of crop contents.

The aim of this thesis was to learn more about the startle response of the Colorado potato beetle. For example, what stimuli trigger it and what factors affect recovery from the immobile state? Is beetle sensitivity and recovery age or gender dependent? Are parameters of the response affected by pre-startle behaviors such as walking, feeding and grooming? Do beetles become more sensitive or habituate with repeated stimulation? Though responses to danger signs (e.g. predators) do not tend to habituate (Thompson et. al. 1973), some organisms show a decremental response to alarm stimuli. For example the cockroach escape response decreases with repeated stimuli (Baxter, 1907). Similarly, with repeated stimuli, praying mantids show a decrease in their fright response to birds (Balderrama and Modonado, 1971). Hungry animals tend to be more impulsive (Snyderman, 1983). Does the physiological state of beetles affect recovery? Lastly I used ablation experiments and histological observations in an attempt to determine what sense organ is involved in mediating the startle response in Colorado potato beetle.

Figure 1. The Colorado potato beetle in thanatosis with legs extended (above) and withdrawn (below).

CHAPTER 2: MATERIALS AND METHODS

As mentioned in the introduction, the Colorado potato beetle startle response was characterized by complete cessation of activity. A high percentage of beetles responded to startle stimuli and they stayed on the substrate (e.g. leaf) during the startle response. As there was little variation in percent response under most conditions, recovery from the immobile phase (recovery time) was the more useful variable to study. Recovery from the thanatotic state was usually preceded by either antennal or leg movement, and occasionally by movement of mouthparts. This chapter deals with the development of methods used to explore this response in detail.

Insects and Plants

Life cycle of beetle

The beetle lays its eggs on either the leaves or stems of *Solanum tuberosum* L. (potato). The eggs hatch in 7 days and there are usually 4 larval instars. The larval period lasts up to 15 days depending on climatic conditions. Pupation takes place in the soil, and adult beetles emerge one to two weeks later. Thus the average life cycle is about 32 days (Jacques, 1988).

Rearing method

Adult beetles from a laboratory colony established in May, 1990 were used for the experiment. Photoperiod was maintained at 16h:8h:L:D and temperature at 25 $\pm$ 1 ^{o}C. Room lighting was provided by full-spectrum fluorescent fixtures. Breeding adults were kept in large aquaria (61 x 32 x 38 cm^3) and given cuttings of *S. tuberosum* and occasionally *S. dulcamara*. Eggs were collected from adult breeding aquaria and placed in Petri dishes until hatching, or just prior to hatching and then placed on potted potato plants. Third instar larvae were transferred from the potted plants to special nurseries made of two plastic containers. The bottom container had a

3-5cm layer of sand and peat. Fresh cut *S. tuberosum* plants in a jar of water, held in place by foam, were placed in the nursery for larval food and pupation occured in the sand/peat mixture. Newly emerged adults were harvested twice daily and placed in a separate aquaria for experiments. For additional details, refer to Mitchell and Harrison (1984).

S. tuberosum plants were grown from tubers. Plants were grown in the greenhouse under high intensity lighting for 16h per day. Fertilization was 20:20:20; NPK once per week or as needed. Temperatures ranged from 18 °C - 28 °C.

Stimulus Quality

Three different stimuli were tested for their ability to produce the startle response viz.: light, mechanical and sound. A camera flash presented to beetles moving on a host plant in a darkened room had no effect. The impact of weights on the table supporting the host plant and the beetle did cause a startle response. To distinguish between the sound and vibration components of the falling weight, weights were dropped on a second similar table near, but not touching, the one supporting the insect and its host plant. Beetles did not respond unless the weight was dropped on the table supporting the host plant and beetle. Sound produced by slapping wooden or metallic rods against each other and also popping balloons did not cause a startle response. Of the three stimulus qualities investigated, mechanical disturbance, presumably vibration, was the only one that led to the startle response. In the experiments to follow, aspects of the vibratory stimuli as well as various parameters of beetle condition are considered.

Materials and Methods Common to all Experiments

Given the importance of mechanical vibration shown in the previous section, two major methods of startling beetles were developed for the experiments described in this thesis. In all cases beetles were either on a host plant or on an artificial silk plant with broad leaves and purple flowers similar to the host. Both young (1-7 days) and old (8-14 days) beetles were used. This age range was used because old beetles (> 2 weeks) were more difficult to experiment upon since they kept walking off the substrate. For all the experiments, 'The Observer' software package (Noldus, 1991), was used in the data collecting session and data obtained were analyzed using GLM ANOVA. Difference between means was tested by either Fisher's LSD (p=0.05%) or Duncan's Multiple Range Test (DMRT).

Startle Method I (Sinkers)

Sinker sizes of 0.5, 1.0, 1.5, 2.0 and 5.0 with corresponding weights of 14, 32, 43, 59 and 135 grams respectively were used (Figure 2). Individual beetles were placed on a cutting of potato plant held in a jar of water supported on a metal frame table with a ¾" plywood top. The sinkers were dropped from a height of 60 cm to strike the table top 16 cm from the jar. Unless otherwise indicated, an inter-startle interval (i.e. the time between recovery and the next startle) of 5 min was used for experiments employing this method.

Startle Method II (Frequency)

To determine the nature of vibrations produced by the falling sinkers on the table top, a galvanometer was adapted to measure leaf vibrations. The galvanometer was allowed to touch the leaves of cut potato plant mounted as in method I and the voltage signals generated when weights were dropped were displayed on a Tektronix oscilloscope, recorded and subsequently digitized. When the weight struck, the leaf

moved in the form of a sine wave with diminishing amplitude and a frequency of 110 Hz. The frequency components of the movement were independent of the different weights used. To simulate this movement a low frequency "Woofer" speaker was subsequently employed.

Figure 3 shows the experimental set up used in method II. A 12", 4 Ohm (Woofer) speaker was fitted with a cardboard platform on which a potted artificial plant was secured with a plastic sealing compound, terostat II (Teroson, Heidelberg). The artificial plant was used to reduce the number of potential pre-startle behaviors to walking, grooming and resting. The speaker was connected to a Heathkit solid state amplifier (model AA-18) and a 3025 sweep function generator was used to generate different frequencies. The amplifier and a 1900A multi-counter were both connected to the sweep function generator. The multi-counter gave an accurate measurement of the number of cycles per second. A Potter & Brumfield timer was connected to the trigger input on the frequency generator to allow for accurate control of stimulus duration. In addition to simulating the movement caused by the falling sinker, this experimental set-up allowed for greater control of frequency, stimulus duration and pre-startle activity compared to method I. In experiments conducted with this method, beetles were given 5 min to settle and the inter-startle interval was also 5 min, unless stated otherwise. Experiments reported in this thesis were conducted using either one or both of these two methods. Method I was used to address some fundamental questions such as the effect of age, sex and pre-startle behavior on recovery time and method II was used to confirm and extend results from experiments performed using method I.

Displacement

The volume control of the amplifier was used to vary speaker displacement. Three levels were used: 1, no perceptible displacement of the cardboard support, 2, a displacement of 0.6 mm and 3, a displacement of 1.3 mm. Displacement of the

support platform was measured with a micro-manipulator graduated in millimeters. Since the speaker displacement was transmitted to the entire plant and its container, movement of leaves on the artificial plant was assumed to be directly related to speaker displacement.

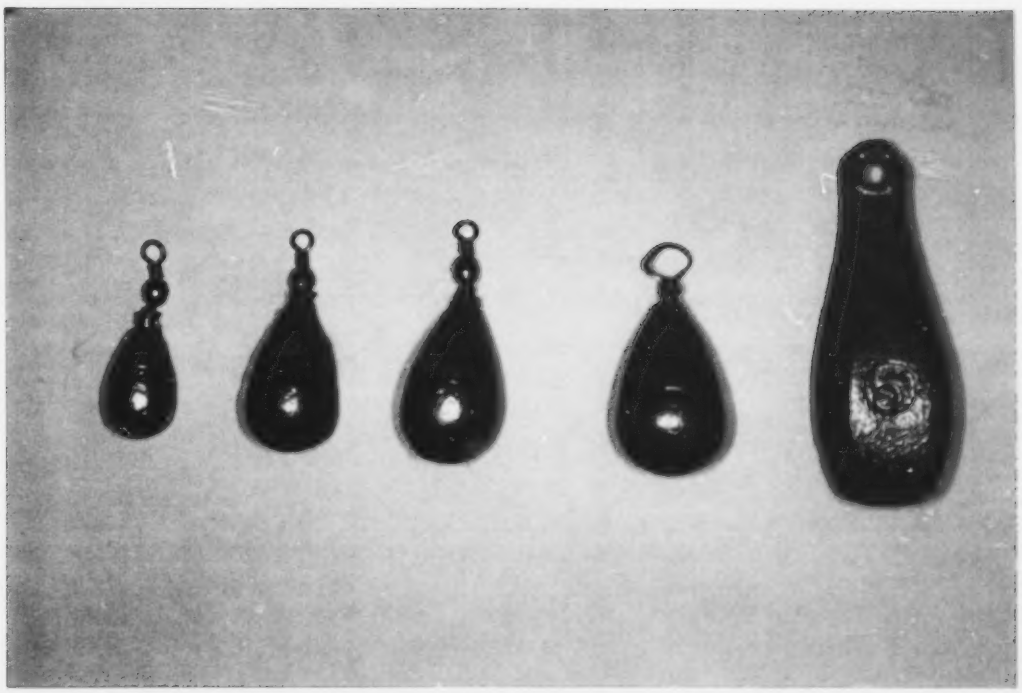

Figure 2. From left, sinker sizes in increasing order of magnitude. 0.5, 1.0, 1.5, 2.0 and 5.0 with corresponding weights of 14.2, 32, 43.4, 59.5 and 134.6g.

Figure 3. Experimental set up. A = 12" speaker fitted with a cardboard platform, B = Potter and Brumfield timer, C = Heathkit solid state amplifier (model AA-18), D = 1900A multi-counter and E = 3025 sweep function generator. The speaker was connected to the amplifier. The amplifier and multi-counter were both connected to the sweep function generator at high and low outputs respectively. The multi-counter gave an accurate assessment of frequency. The timer was connected to the trigger input outlet on the sweep function generator to allow for accurate measurement of stimulus duration.

CHAPTER 3: PHYSICAL AND BIOLOGICAL PARAMETERS AFFECTING THE STARTLE RESPONSE

With the exception of ablation experiments and histological observations, which are dealt with in the subsequent chapter, this chapter deals with experiments conducted to elucidate various features of the startle response.

Physical Parameters

Stimulus Strength

Beetles were allowed 10 mins to settle and startled three times with one of the weights. The three startle attempts were analyzed separately. A total of 68 beetles were used for this experiment including young and old beetles of both sexes.

Figure 4 shows the effect of stimulus strength on recovery time. For all 3 startles, the mean recovery times due to the different stimuli were not significantly different (Table 1), though sinker 1 gave the highest mean recovery time. Sinker 1 also gave the lowest percentage of non-responses i.e. 20.2 % (Table 2). Sinker 2 was also a very effective stimulus (23.5% non-responses). The least effective stimulus, measured by percentage non-response, was sinker 1.5 (48.3%) (Table 2).

Gender and Age Effects

Gender and age effects were investigated under the startle regime described above and figures 5 and 6 show the effect these two variables on recovery time respectively. For all three startles, the mean recovery time for males was not significantly different from that for females. Similarly, age did not affect recovery time (Table 1).

Stimulus Frequency

Employing stimulus method II, frequencies of 10, 30, 50 and 110 Hz were used in a randomized design. 9 beetles of both sexes were tested. Each beetle was startled using the four frequencies, presented in a random order. Each startle frequency was replicated three times. Beetles were startled when they were either walking or grooming.

Figure 7a shows the effect of frequency on recovery time. Mean recovery time increased with frequency up to 30 Hz and decreased thereafter. Thus the maximum mean recovery time of 187 $\pm$ 40 sec was obtained at a frequency of 30 Hz and the minimum mean recovery time of 43 $\pm$ 8 sec at a frequency of 110 Hz. These two recovery times were significantly different (Fisher's LSD $p<0.05$). However, the mean recovery times for 50 Hz and 110 Hz were not significantly different (Fisher's LSD $p>0.05$). With respect to percent response, 30 Hz was the most effective stimulus (Figures 7b).

Interestingly, a non-response rate of 50% was obtained at a frequency of 10 Hz, but beetles that did respond to this frequency had a recovery time that was not significantly different from recoveries from either 30 or 50 Hz stimuli (Fisher's LSD $p>0.05$).

Stimulus Duration

A stimulus frequency of 30 Hz was used at five different durations in a randomized design. The durations used were 0.1, 0.5, 1.0, 2.0 and 4.0 sec. 7 beetles of both sexes were used for this experiment. Each beetle was startled with all 5 durations in a randomized order. The inter-startle interval was 5 min.

Figure 8 shows the effect of stimulus duration on recovery time; it was the same over all durations tested ($F = 1.24$ d.f = 4,30 $p>0.05$).

Effect of Displacement on Recovery Time

Each beetle was introduced onto an artificial plant, allowed 5 min to settle and startled using two displacements (0.6 and 1.3 mm) at a frequency of 30 Hz. 3 determinations were made for each displacement and the average recovery time was used in the analysis. 10 beetles of both sexes were used.

Figure 9 shows the effect of degree of displacement of the support medium on recovery time. The mean recovery time using a displacement of 1.3 mm was significantly greater than that for 0.6 mm i.e. 392 $\pm$ 75 sec vs. 148 $\pm$ 25 sec ($F = 8.46$. $d.f = 1,10$ $p<0.05$). On the basis of individual recovery time, 80% of the beetles tested showed this same trend of long recovery times with the displacement of 1.6 mm (Figure 10).

Beetle Behavior and Condition

Sensitization (i.e. increase in responsiveness towards a repeated stimulus) and habituation (decrease in response to a monotonous stimulus) occur in nature. In the following experiments I tried to determine if beetles would become more sensitive or habituate to repeated presentations when inter-startle intervals of less than 5 min were used, whether there is an effect of pre-startle activity on recovery time and whether a startle stimulus given, for example 10 sec after the beetle enters the immobile state, will prolong recovery time.

Sensitization and Habituation

Since stimulus strength did not affect recovery time, sinker number 2 (method I) was used for the first of two experiments. Beetles were allowed 10 min to settle when introduced onto a cutting of potato plant. Each beetle was then startled 8 consecutive times and each startle after the first one was presented at the first signs of recovery

(recovery from the immobile state was usually preceded by either antennal wiggle or leg movement and occasionally by movement of mouth parts). 22 beetles of both sexes were used for this experiment. In a second experiment using method II, a slightly different approach was taken in that the number of startles was increased from 8 to 22 and an inter-startle interval of 1 min was used instead of startling at the first sign of recovery. 13 young beetles of both sexes aged 3-7 days were used for this experiment. Beetles were startled with a frequency of 30 Hz and a displacement of 1.3 mm.

Figure 11 shows the effect of eight repeated startles with sinkers on recovery time. Though the mean recovery times for startles 1 through 8 were not significantly different (Fisher's LSD $p>0.05$), there was a trend of sensitization followed by habituation. Variability increased with the length of recovery time. Neither habituation nor sensitization were pronounced. I tried to confirm the above results using method II.

Figure 12 shows the effect of repeated presentations on recovery time using method II. On average, beetles became more sensitive to repeated presentations up to startle number 6. Thereafter there was a decrease in sensitivity. This same trend was exhibited by a large number of individual beetles (Figure 13 & 14). There was a linear relationship between startle number and the number of non-responses i.e. the number of non-responses increased with repeated presentations (Figure 15).

The trend of sensitization, followed by habituation was clear in both experiments. In the second experiment, the mean recovery times for startles 1 and 3 were significantly lower than for startle 6 (Fisher's LSD $p<0.05$). The other means were not different. Since the number of non-startles increased with repeated presentations, the number of recovery time measurements was quite low after the 8th startle. This accounts for the lack of significant difference, for example, between startles 6 and 17.

Sensitivity during Recovery Phase

First Experimental Design

In this experiment, I tested the effect of a second stimulus presented 10 sec within the immobile phase and compared it with the effect of a single stimulus. These were called 'double' and 'single' startle respectively. Using method I, each beetle was startled three times with sinker 2 after the 10 min settling period. The stimulus sequence for a double startle was as follows: 10 min settling, Startle1(s1a 10 sec s1b), 5 min inter-startle interval, Startle2 (s2a 10 sec s2b)...etc. and that for a single startle; Startle1, 5 min inter-startle interval, Startle 2, 5 min inter-startle interval, Startle 3.

Figure 16 shows the effect of double startle on recovery time with reference to the pre-startle behavior. Though with walking beetles, recovery time more than doubled with the double startle, this difference was not significant due to the large increase in variability associated with the double startle ($F = 2.17$. d.f = 2,21 $p > 0.05$). With feeding beetles, there was only a slight increase in recovery time with double startle.

Second Experimental Design

To obtain a better control of variables, the above experiment was repeated using method II. This method excluded feeders, thus beetles were startled when either walking or grooming. Method II also excluded the inherent variability of stimulus delivery associated with method I. Beetles were startled 3 times with a frequency of 30 Hz, 1.3 mm displacement. Following each initial startle, beetles were either not startled (single startle), startled once (double startle) or startled twice (triple startle) within the immobile state. Within the immobile state, startles were given 10 sec apart. The single, double and triple startles were presented in a random design and each type of startle was replicated 3 times. 5 min were given between replicates. 13 beetles of both sexes were used for the experiment.

Recovery time increased with the number of startles within the quiescent state and was highest for the triple startle which was significantly different from both the single and double startles (DMRT $p<0.05$) (Figure 17). Since recovery time did not show signs of plateauing at 3 startles, another experiment was conducted to find out whether increasing the number of startles within the quiescent state would result in a decreased or a plateau response. The same protocol as above was employed with the exception that two replicates instead of three were made for each startle. 6 beetles were used for this experiment. Startle numbers were 1, 3, 5 and 7. Increasing the number of startles within the quiescent state led to prolonged recovery times, with no sign of a plateau effect at even the highest startle number (Figure 18).

Pre-Startle Behavior

Beetles were startled when they were either walking, feeding or grooming. Data from three previously described experiments using method I, were analyzed based on pre-startle behaviors, and the results are presented here. Data from the experiment on stimulus strength were pooled, since sinker weight did not affect recovery time. When analyzed for the effect of pre-startle behavior, the three pre-startle behaviors did not appear to have any differential effect on recovery time (Figure 19). (Startle 1; $F = 0.18$, $d.f = 2,65$ $p>0.05$, Startle 2; $F = 1.03$, $d.f = 2,52$ $p>0.05$, Startle 3; $F = 0.94$, $d.f = 2,53$ $p>0.05$).

From the data used in the sensitization and habituation experiment (method I), when beetles were separated based on pre-startle activity, the mean recovery time of beetles startled while feeding was shorter than recovery times of beetles startled while walking. This was true for all 8 startles (Figure 20). The longer recovery time for walking vs. feeding beetles was significantly different, however, only for startle number 1 (Fisher's LSD, $p<0.05\%$). The lack of significant differences for startles 2-8 can be attributed to the increasing number of non-startles with successive trials (Table

3). In contrast to the stimulus strength experiment, there does appear to be an effect of pre-startle activity on recovery time when the stimulus is consistent throughout the experiment.

When data from the sensitivity during recovery phase experiment (method I), were analyzed for the effect of pre-startle activity, it was clear that insects recovered earlier when startled while feeding than while walking or grooming (Figure 21) (Startle 1; $F = 2.17$, d.f = 2,21 $p>0.05$, Startle 2; $F = 1.28$, d.f = 2,23 $p>0.05$, Startle 3; $F = 1.68$, d.f = 2,21 $p>0.05$).

Activity after Startle

I observed that some beetles returned to pre-startle activity upon recovery while others did not. This experiment was designed to detect any pattern in pre and post startle behavior.

Data collected for the stimulus strength experiment, were grouped by pre- and post-startle activity and a summary of the results is given in Table 4. For all three pre-startle behaviors, a majority of beetles returned to the pre-startle activity following recovery. This was most pronounced if the pre-startle activity was feeding ($\chi^2 = 36.63$, $\chi^2_{.05}\{1\} = 3.841$), followed by grooming ($\chi^2 = 10$, $\chi^2_{.05}\{1\} = 3.841$). For the pre-startle behavior of walking, though a majority of beetles returned to the pre-startle activity following recovery, this was not significant ($\chi^2 = 1.8$, $\chi^2_{.05}\{1\} = 3.841$).

Satiation Level

3-day old beetles were starved for 24 hours after which they were startled with a frequency of 30 Hz and a displacement of 1.3 mm. Beetles of both sexes were used and the inter-startle interval was 5 min. Three determinations were made for each

beetle, and the mean recovery time for each beetle was used in the analysis. In the control experiment, a different group of fed beetles of both sexes was tested.

Figure 22 shows the effect of satiation level on recovery time. Fed beetles took a longer time to recover compared to starved beetles, in fact there was a fourfold decrease in recovery time with starvation ($F = 15.67$. d.f = 1,12 $p<0.05$).

Field Collected Beetles

Beetles collected from the field were tested to compare aspects of startle behavior with laboratory reared beetles. Method I was used for this experiment. Beetles were allowed 10 min to settle, startled 3 times with sinker size 2 and the inter-startle interval was 5 min. 10 beetles of both sexes were tested for each group.

Generally, field collected beetles took a longer time to recover from the immobile state compared to laboratory beetles i.e. 54 ± 11 vs. 27 ± 6 sec. The means were significantly different (Fisher's LSD $p<0.05$).

Discussion

The startle response in the beetle was induced by mechanical means i.e. vibrations, and the length of recovery depended upon displacement caused by the vibration. For the frequency range of 10-110 Hz, 30 Hz was found to be the most effective frequency for startling beetles with respect to recovery time and percent response. The honey bee, *Apis mellifera* is known to exhibit a startle response when disturbed by vibrations but the response is induced by much higher frequencies, between 500 and 1000 Hz. (Little, 1962). In the Plecoptera, where drumming of body parts is used to produce vibration for recognition of the sexes, frequency of drumming is more important for recognition than its duration (Rupprecht, 1968). Beetle age, sex and stimulus duration did not affect recovery time.

Beetles were initially sensitive, but later habituated to repeated stimuli. The initial increase might be an adaptation to counter additional threats and thus might reflect a kind of priming for optimal defensive responsiveness. With increasing number of startles, however, beetles learned to "ignore" the stimuli, this leading to habituation (Carew et. al. 1985). Habituation was most clearly demonstrated by the linear relationship between startle number and number of non-responses. The escape response of the cockroach decreases with repeated stimuli (Baxter, 1907). Also when praying mantids are exposed to birds repeatedly, their fright response decreases (Balderrama and Madonado, 1971).

A majority of beetles returned to the pre-startle behavior upon recovery. This suggests that the mechanism mediating the startle response does not affect the post-startle behavior. The percentage of beetles returning to the pre-startle behavior after recovery, was highest for feeding beetles. There was also a fourfold decrease in recovery time with starvation. Carew et. al. 1985 state "for energetic efficiency, the strength and duration of a reflex response should match as closely as possible the 'expected' importance of the stimulus". For starved beetles, presumably feeding is a priority, thus beetles recover earlier in order to search for food while in fed beetles the startle response takes precedence over feeding. Also Snyderman, 1983 has shown that hungrier predators are more impulsive i.e. they are less selective regarding prey.

Startle within the quiescent state prolonged recovery. This demonstrates that beetles are still receptive to the stimulus while in the immobile phase. Field collected beetles were more sensitive to stimuli than their laboratory counterparts. The reduced sensitivity of laboratory beetles might stem from the fact that they have become adapted to various disturbances in the laboratory environment. The laboratory colony has been cultured since May, 1990. I have been able to find out generally about this behavior but as to whether it is actually an anti-predator mechanism in this beetle is yet to be

established. The natural enemies of the beetle are mostly egg and larval predators thus to test this hypothesis is not going to be easy.

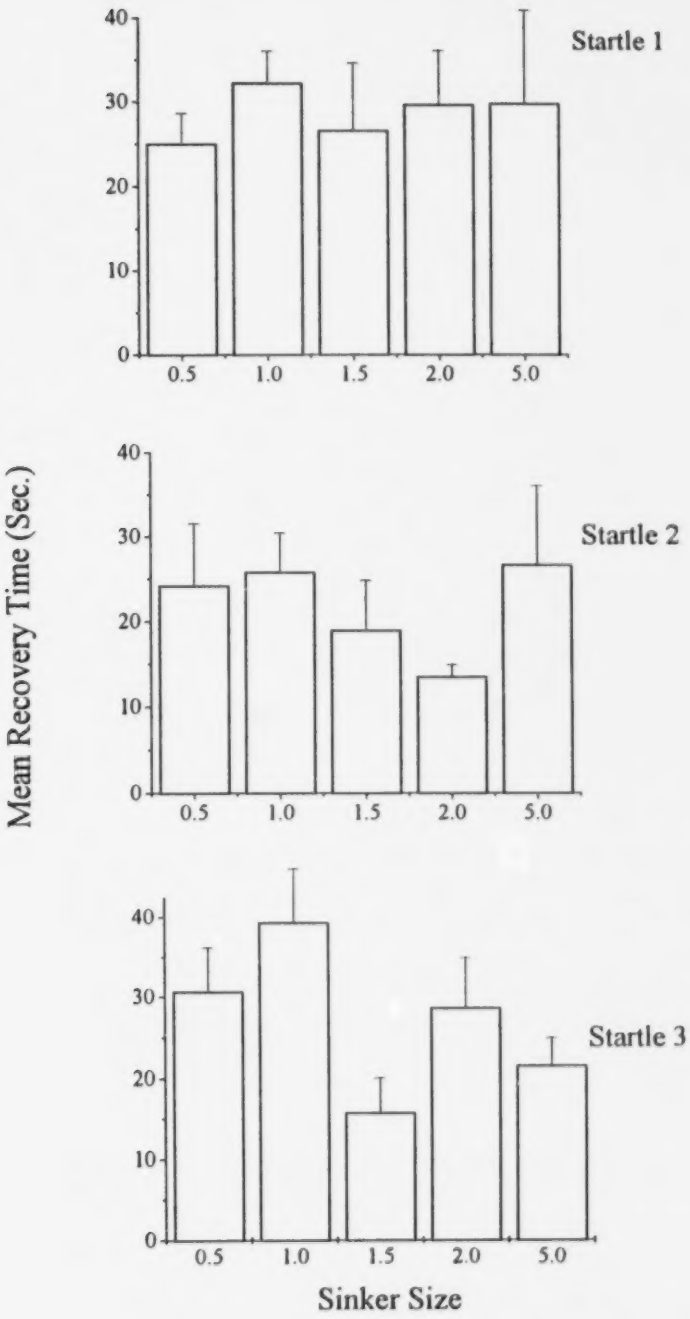

Figure 4. Effect of stimulus strength on recovery time. Entries are means ± standard error. For all three startles; mean recovery time was not affected by stimulus strength (Fisher's LSD $p > 0.05$). N = 68.

	STARTLE NUMBER					
	1		2		3	
VARIABLE	F	d.f	F	d.f	F	d.f
Stimulus Strength	0.22	4,63	1.10	4,50	1.25	4,51
Gender	1.68	1,66	0.47	1,53	2.25	1,54
Age	0.01	1,66	0.35	1,53	0.04	1,54

Table 1. F-values and d.f for stimulus strength, gender and age. The three variables did not have any effect on recovery time ($p > 0.05$).

Sinker No.	No. of Startles	No. of non-responses	% non-response
0.5	39	7	43.6
1.0	84	17	20.2
1.5	60	29	48.3
2.0	51	12	23.5
5.0	30	9	30.0

Table 2. Percentages of non-response for different stimuli. Total number of startles for startles 1-3 was calculated and the number of non-responses for each total was used to obtain percentages.

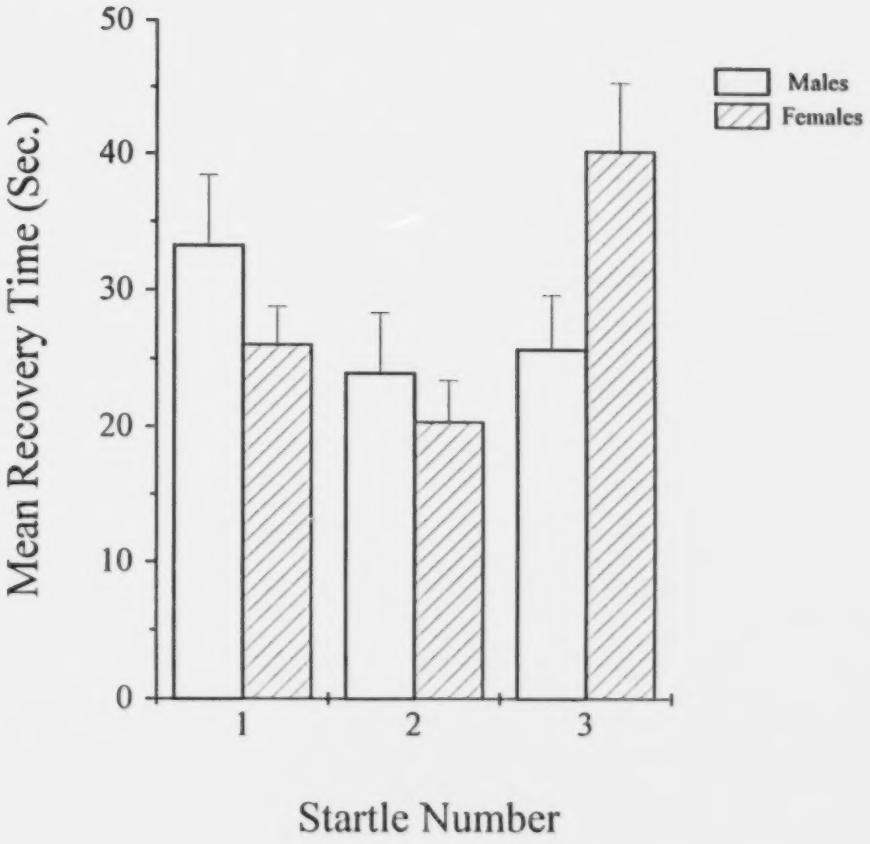

Figure 5. Effect of gender on recovery time. For all three startles, gender did not affect recovery time (Fisher's LSD $p > 0.05$). Entries are means ± standard error. N = 68.

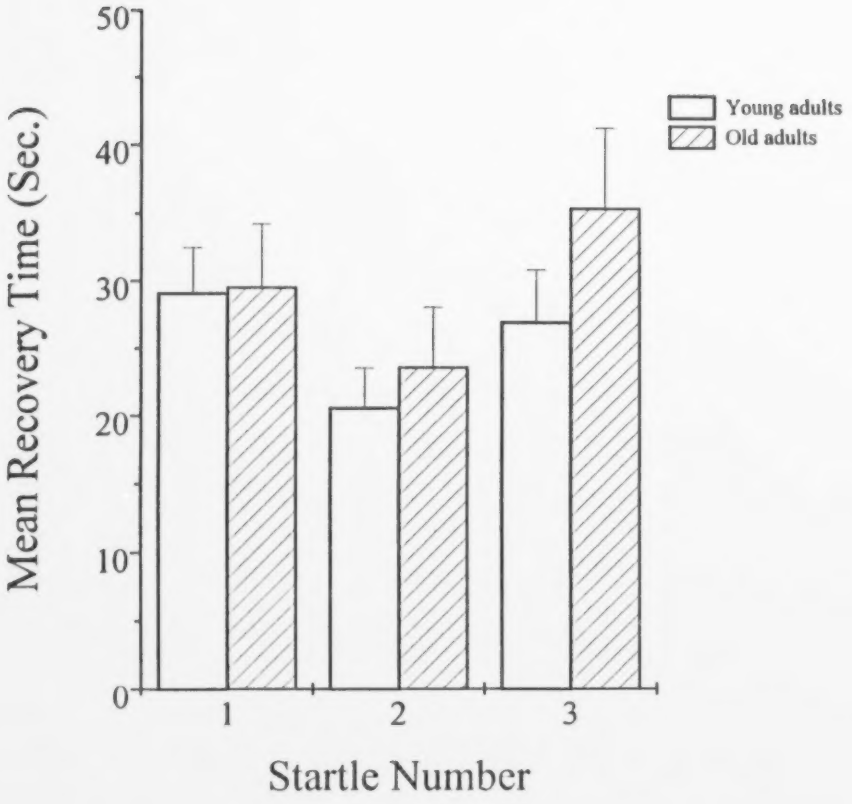

Figure 6. Effect of age on recovery time. Entries are means ± standard error. There is no difference in recovery times between males and females for all three startles (Fisher's LSD $p > 0.05$). N = 68.

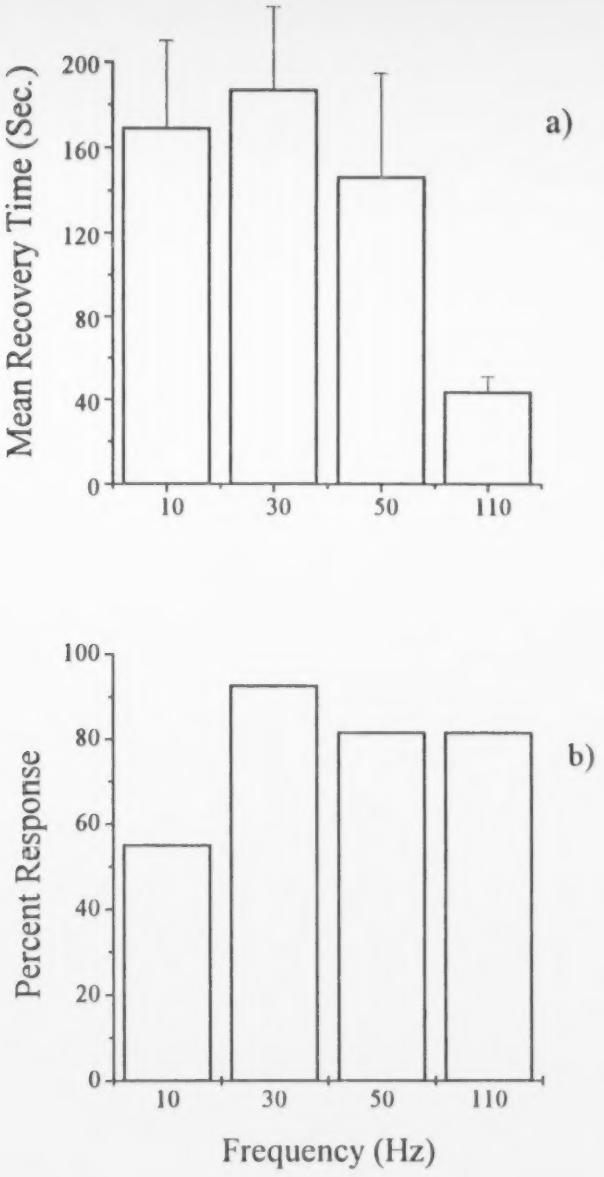

Figure 7. a) Effect of stimulus frequency on recovery time. 30Hz gave the longest recovery time and the greatest percentage of responses. Recovery time due to 110Hz significantly differed from that due to 10 and 30Hz (Fisher's LSD $p<0.05$). $N = 9$. b) Percentage of responses obtained using the different frequencies.

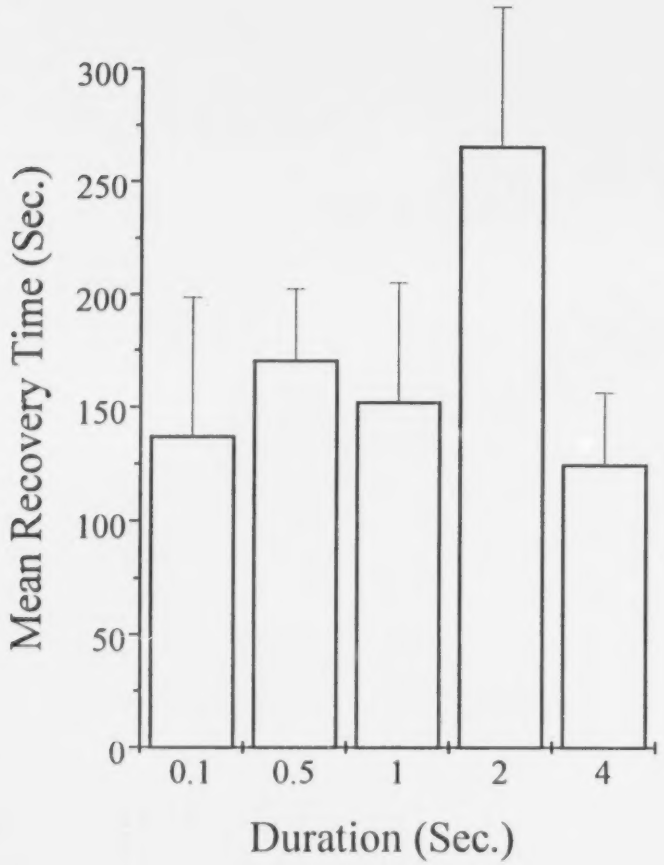

Figure 8. Effect of stimulus duration on recovery time. The duration of 2 gave the highest recovery time. Entries are means ± standard errors. N = 7.

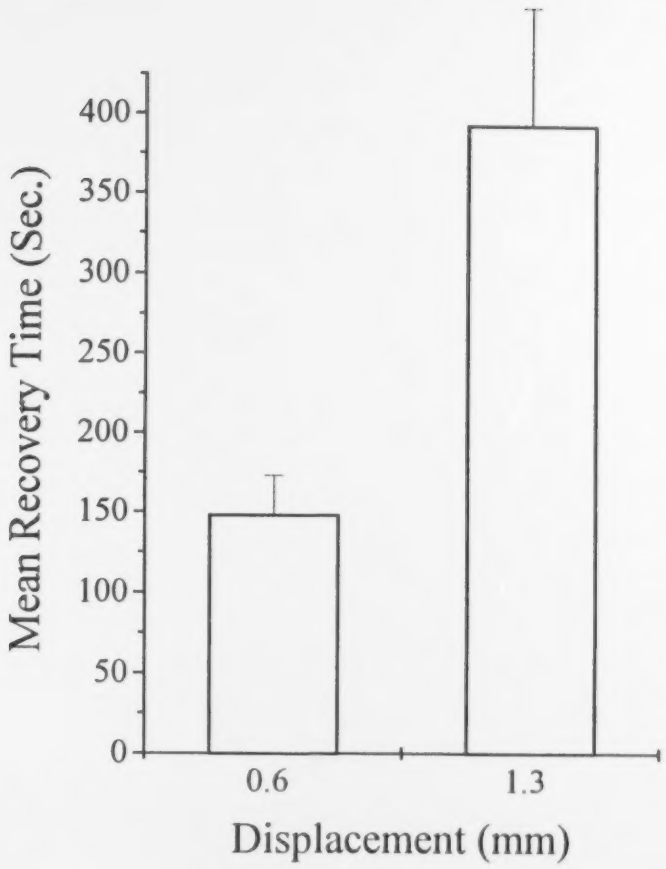

Figure 9. Effect of displacement on recovery time. The recovery time due to displacement of 1.3mm is significantly different from that due to 0.6mm ($F = 8.46$. $d.f = 1,10$ $p<0.05$). Entries are means ± standard error. $N = 10$.

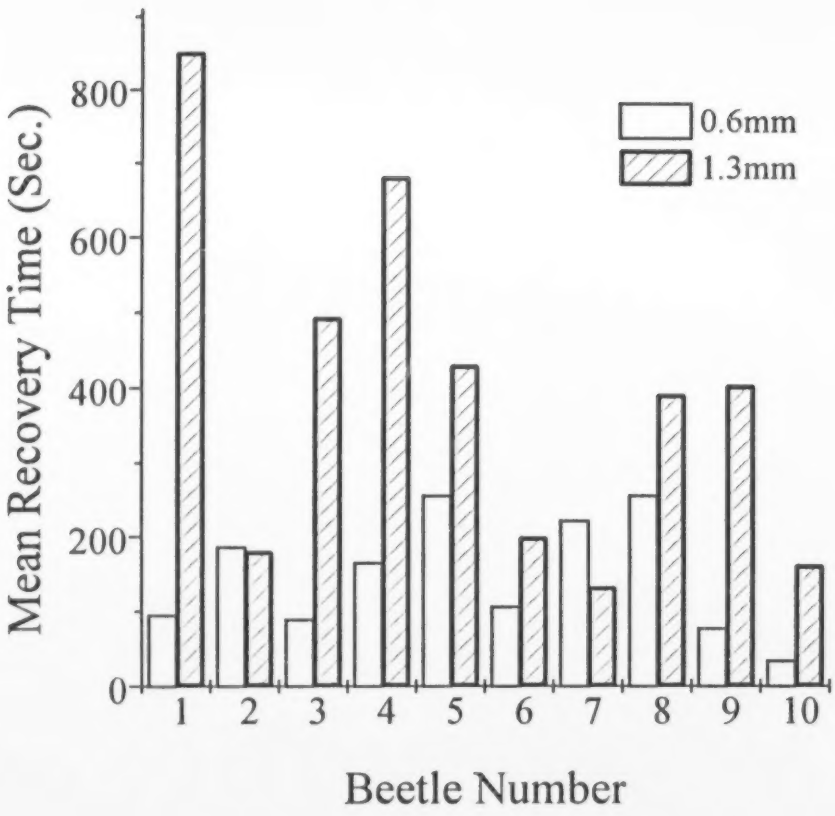

Figure 10. Graph showing the effect of displacement on individual recovery times. Eighty percent of beetles showed a trend of longer recovery times with a displacement of 1.3mm.

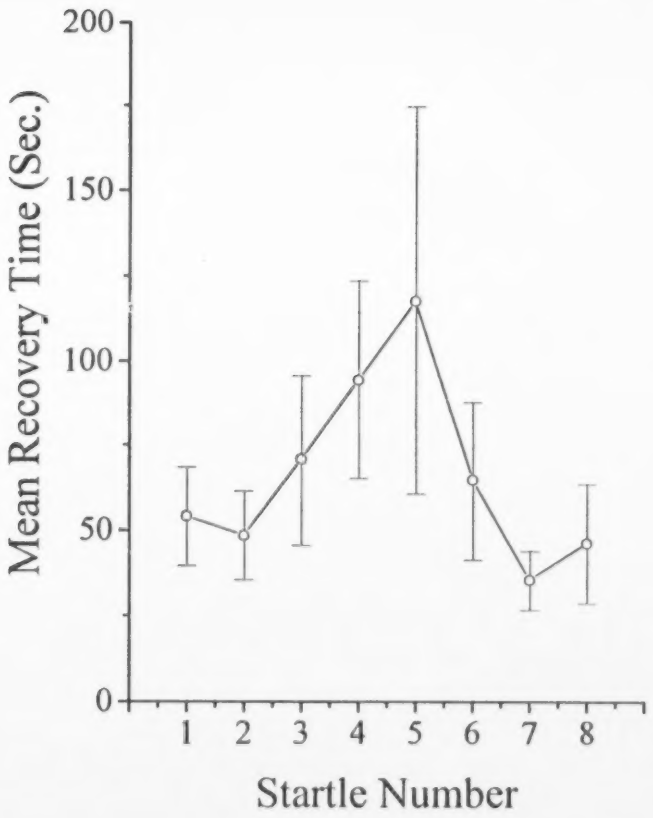

Figure 11. Effect of repeated presentations on recovery time. There was a trend towards sensitization followed by habituation. The mean recovery times for eight startles were not significantly different from each other (Fisher's LSD $p>0.05$). Entries are means ± standard error. N = 22.

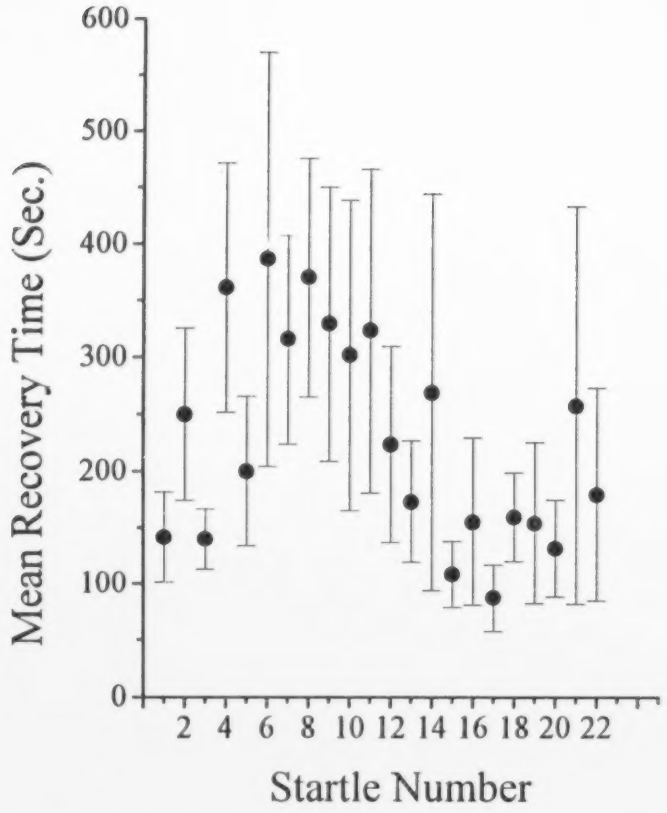

Figure 12. Effect of repeated presentations on recovery time. There is a trend of sensitization, followed by habituation. Recovery times for startles 1 and 3 are significantly different from that due to startle 6 (Fisher's LSD $p<0.05$). Entries are means ± standard error. N = 13.

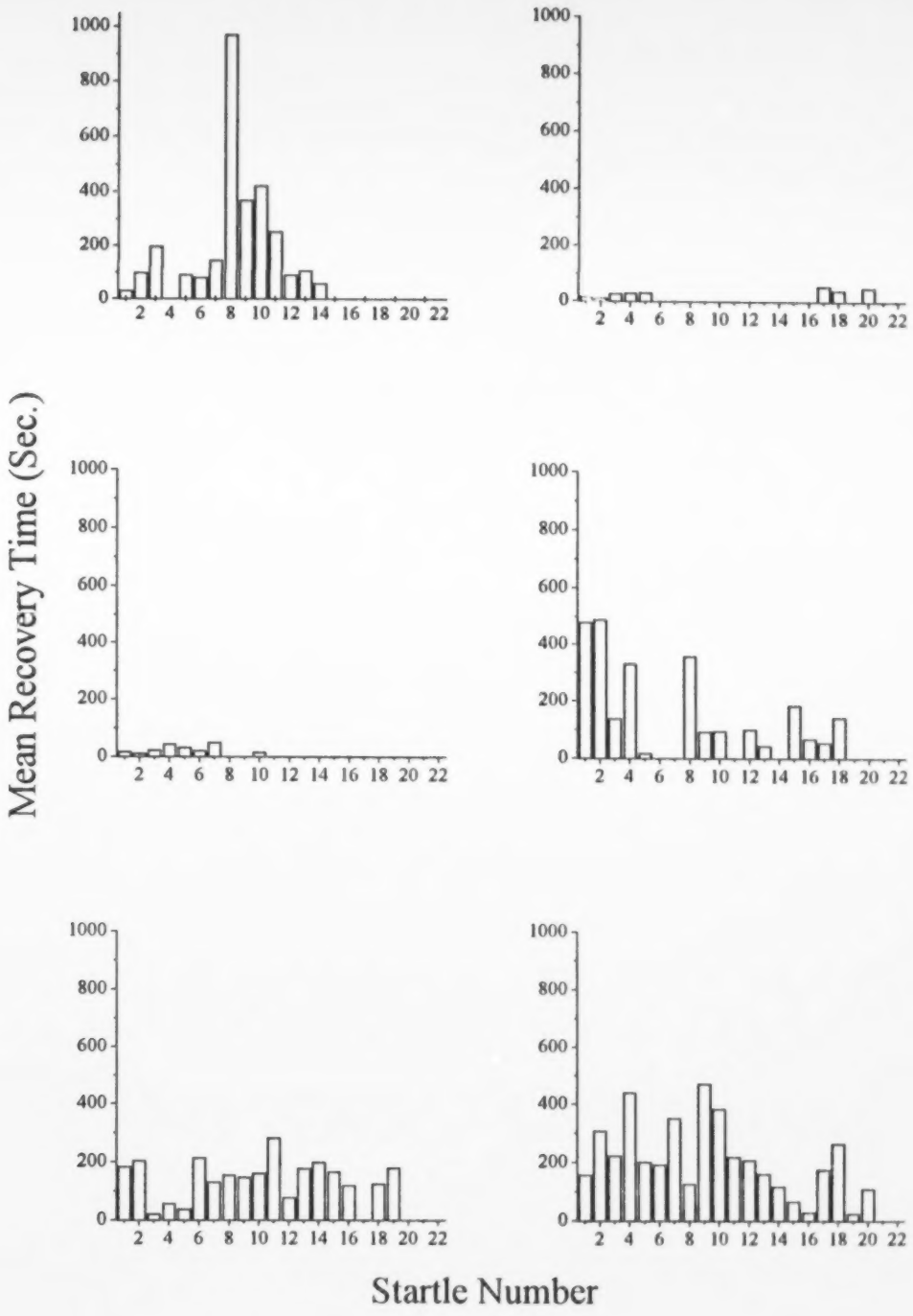

Figure 13. Effect of repeated presentations on individual recovery times. Most beetles showed a trend of sensitization, followed by habituation. N = 13.

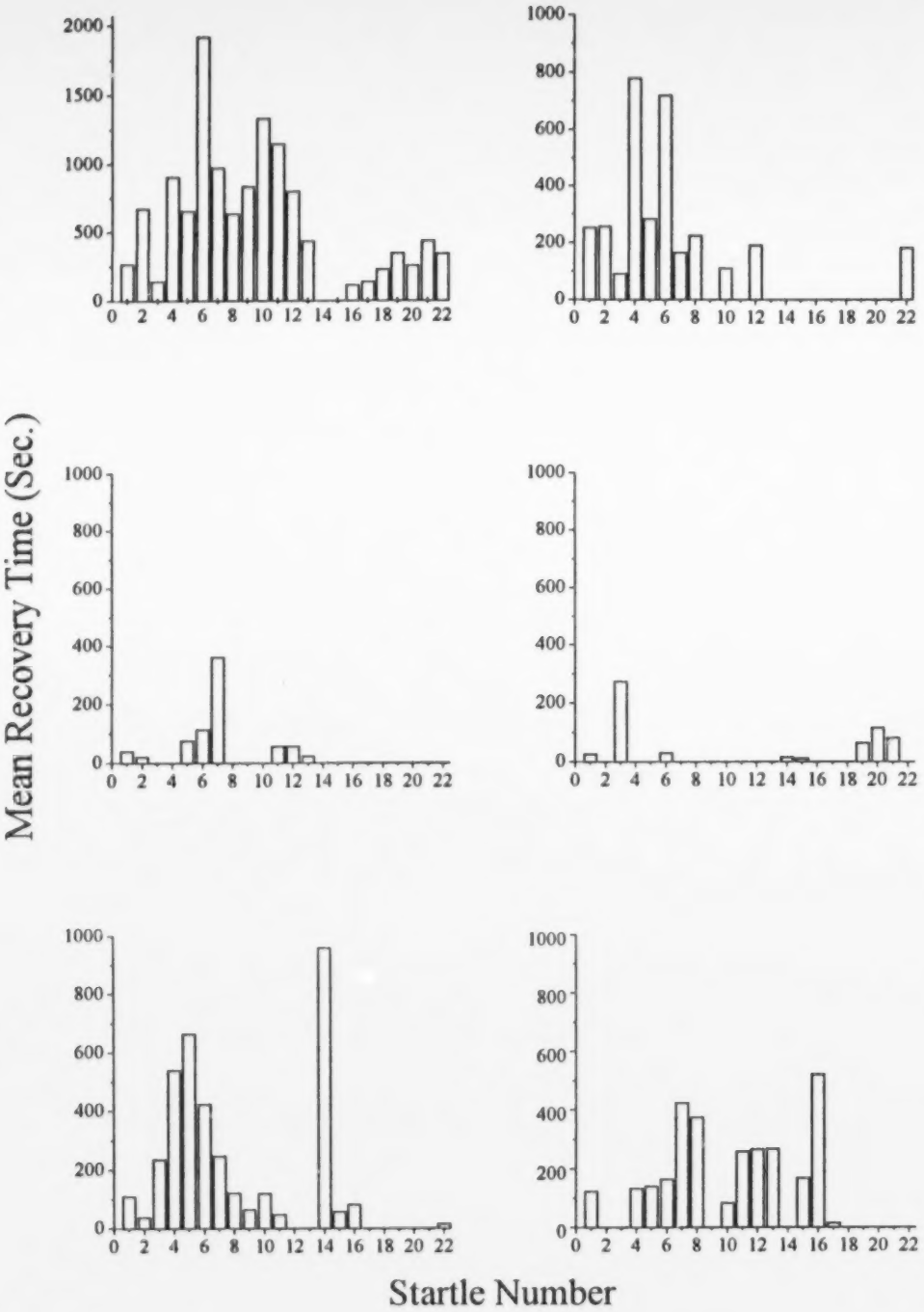

Figure 14. Effect of repeated presentations on individual recovery times- continued.

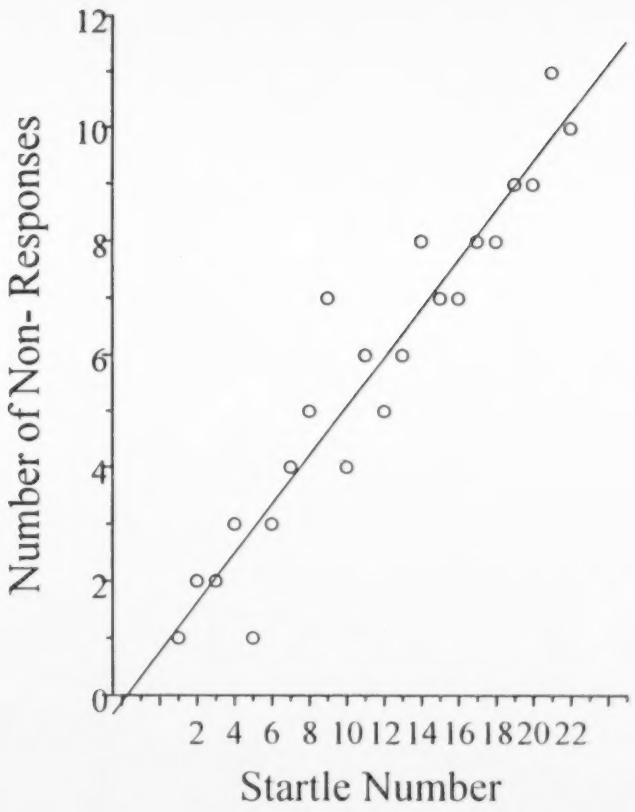

Figure 15. Graph showing the relationship between number of trials and number of non-responses. $y = 5.79 + 3.33x$. $r = 0.95$.

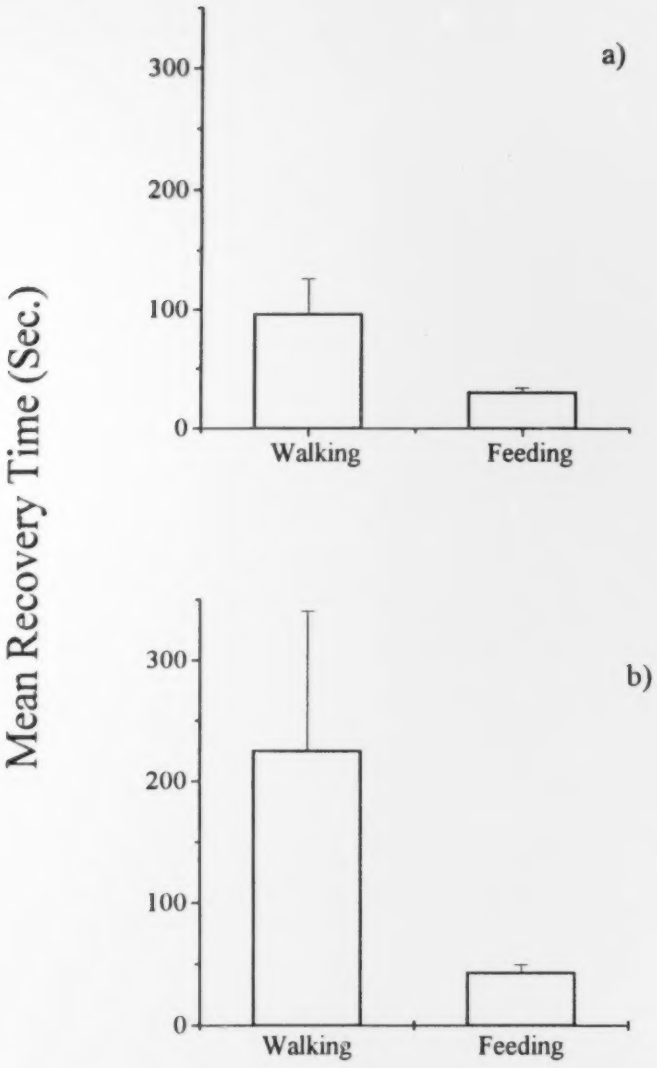

Figure 16. Sensitivity during recovery phase. a = Single startle, b = Double startle. Beetles took longer to recover if startled when walking compared to feeding. Though startle within the recovery phase prolonged recovery this was not statistically significant for both pre-startle behaviors (Fisher's LSD $p > 0.05$). Entries are means ± standard error. N = 24.

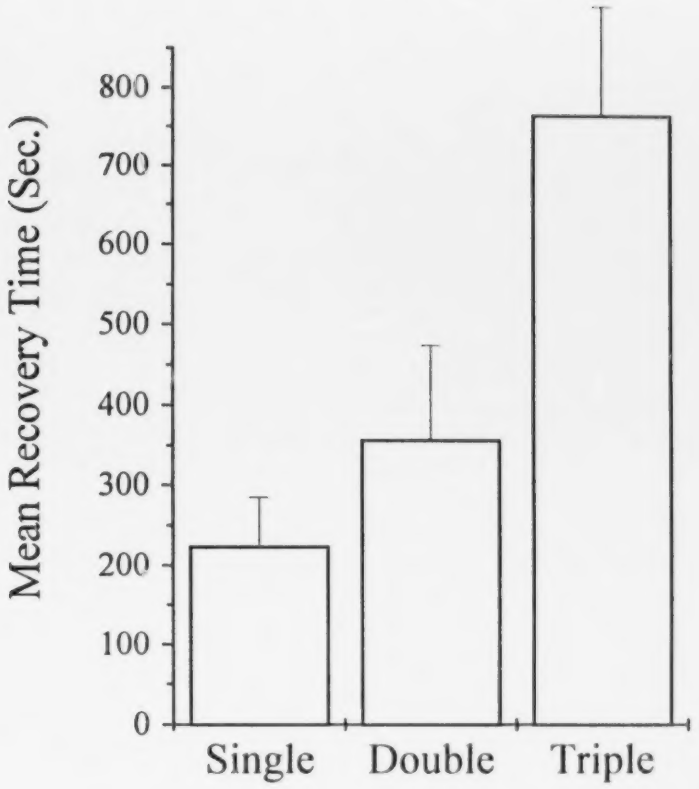

Figure 17. Effect of single, double and triple startles on recovery time. Startle within the immobile state prolonged recovery time. The recovery time due to triple startle was significantly different from that due to both single and double startles (Fisher's LSD $p < 0.05$). Entries are means ± standard error. N = 13.

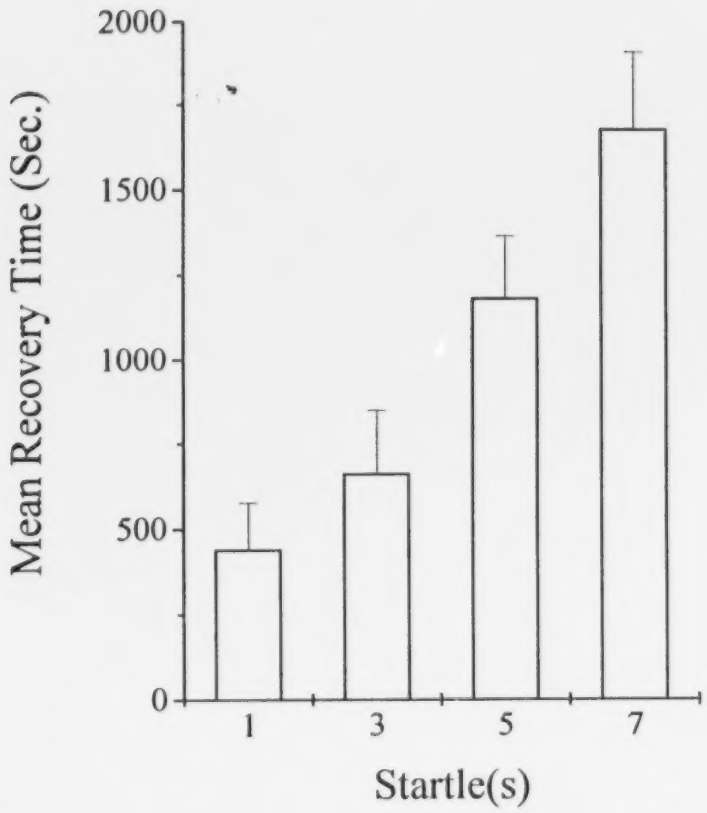

Figure 18. 1, 3, 5 & 7 startles. Recovery time increased with increasing startles within the quiescent state. Mean recovery time due to 7 startles was significantly different from those due to 1 & 3, and the mean recovery time due to 5 startles was also significantly different from that due to 1 startle (Duncan's Multiple Range test $p < 0.05$). N = 6.

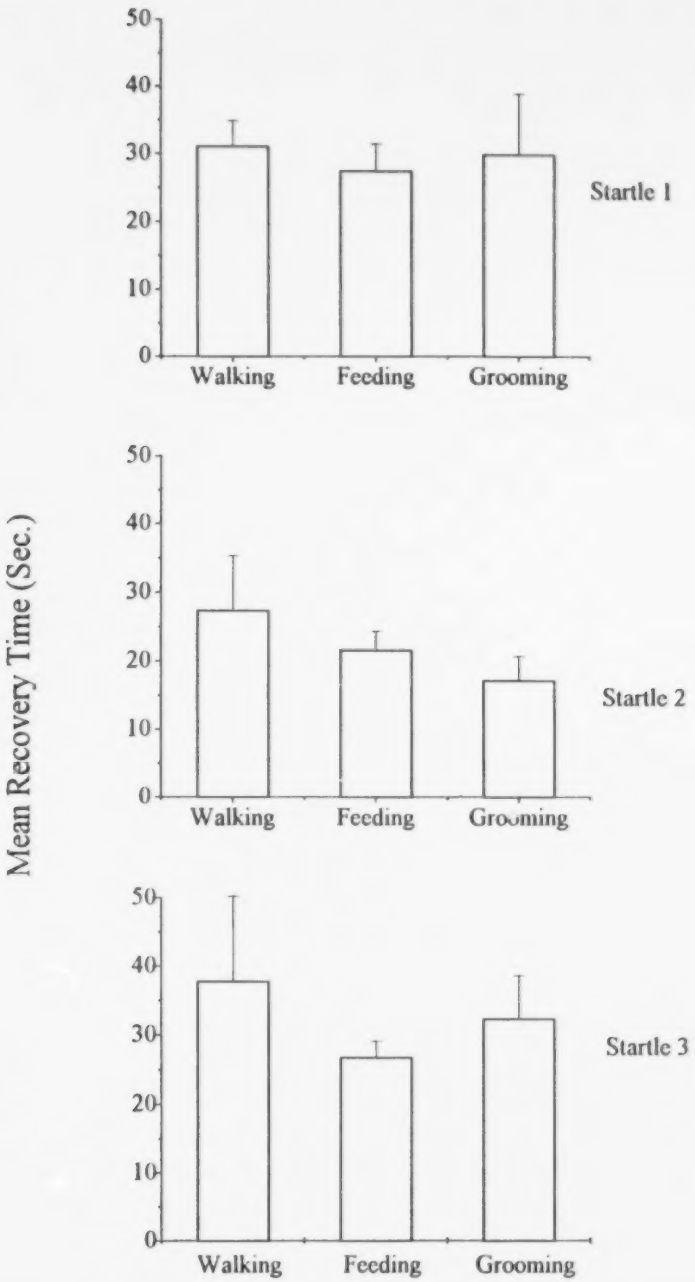

Figure 19. Effect of pre-startle behavior on recovery time. Beetles were grouped based on their pre-startle activity. Pre-startle behavior did not affect recovery (Fisher's LSD $p > 0.05$). There was however, a trend of feeding beetles recovering earlier than walking beetles. Entries are means ± standard error. N = 68.

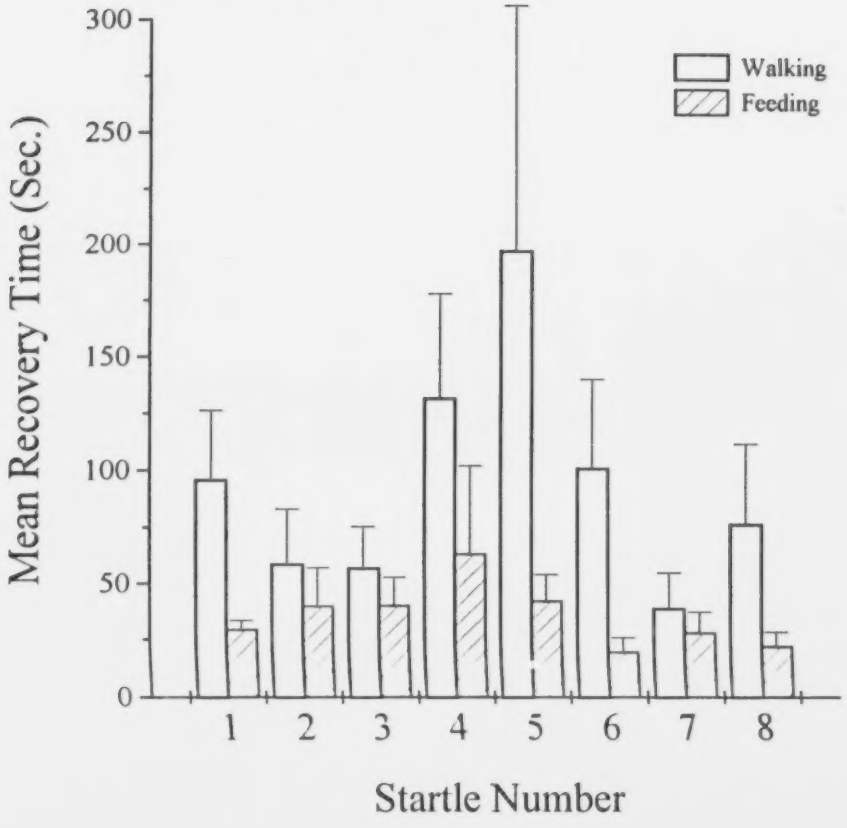

Figure 20. Pre-startle effect on recovery. For all eight startles, when data was analyzed based on pre-startle behavior, beetles recovered earlier if startled when feeding than when walking. Mean recovery times for walking and feeding in startle 1 were significantly different (Fisher's LSD $p<0.05$). N = 22.

Startle Number	Non-Response
1	2
2	2
3	2
4	5
5	4
6	5
7	9
8	10

Table 3. Table showing the number of non-responses with repeated presentations. Twenty-two beetles were used for the experiment. The non-responses shown are out of a total of 22 startles.

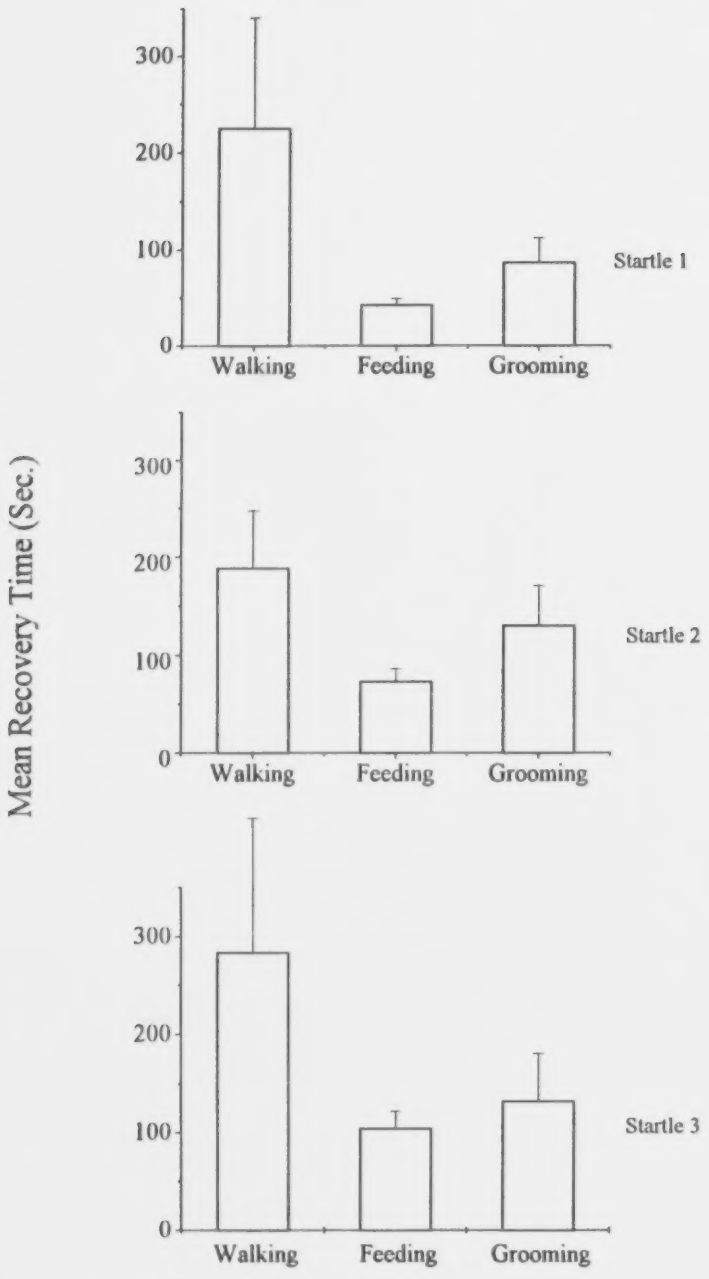

Figure 21. Effect of pre-startle behavior on recovery time. For all three startles, there was a tendency for beetles startled while feeding to recover earlier than when startled while either walking or grooming (Fisher's LSD $p>0.05$). Entries are means ± standard error. N = 24.

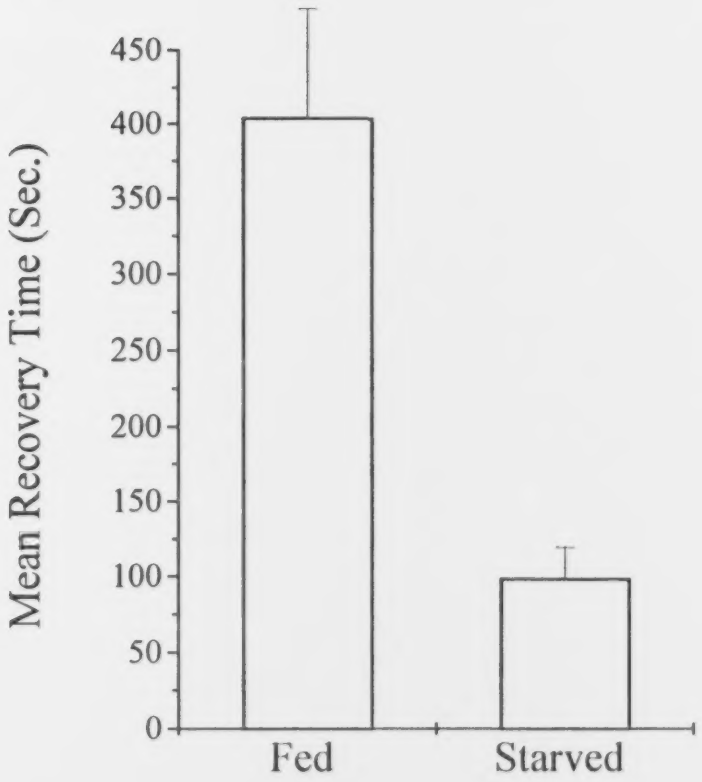

Figure 22. Effect of satiation level on recovery time. There is a fourfold decrease in recovery time with starvation. ($F = 15.67$. d.f = 1,12 $p<0.05$). Entries are means ± standard error. n = 7.

WALKING

Startle Number	Walking →Walking	Walking →Other	Total
Startle 1	14	12	26
Startle 2	5	6	11
Startle 3	8	0	8
Total	27 (60%)	18 (40%)	45

FEEDING

Startle Number	Feeding →Feeding	Feeding →Other	Total
Startle 1	20	8	28
Startle 2	23	1	24
Startle 3	19	2	21
Total	62 (84%)	11 (15%)	73

GROOMING

Startle Number	Grooming →Grooming	Grooming →Other	Total
Startle 1	8	1	9
Startle 2	14	2	16
Startle 3	8	7	15
Total	30 (75%)	10 (25%)	40

Table 4. Beetles were grouped based upon behavior before and after startle. For all three pre-startle behaviors, two out of every three beetles returned to the behavior before startle upon recovery.

CHAPTER 4: PERCEPTION OF VIBRATION

Chordotonal organs serve various functions. They are widespread in the body and they include the Johnston's organ (in the pedicel, sensitive to movements in the antennal flagellum), the subgenual organs (located at the proximal end of the tibiae) and tympanal organs which are involved in hearing (Borror et. al., 1989). In some insects, subgenual organs are known to be sensitive to vibrations of the substrate on which the insect rests (Wigglesworth, 1966). In addition, eyes of insects are especially well adapted for movement detection (Chapman, 1982). It is possible that the vibrations might cause apparent movement of the background and this might lead to startle behavior. Ablation experiments were conducted in addition to the use of histological methods to determine if the beetle has chordotonal organs in the legs, and if so, if these organs are involved in vibration detection. Antennectomy and blinding were also used to ascertain the role of Johnston's organ and vision.

Antennectomy

This experiment was designed to determine if the antennae perceive the mechanical stimulus. First, beetles with their antennae intact were startled with a frequency of 30 Hz and a displacement of 1.3 mm. Subsequently their antennae were removed with iris scissors and they were given 24h to recover. The beetles were startled again after the 24h period. Both unoperated and operated beetles were startled 3 times and the mean recovery time for each condition was calculated.

Figure 23 shows the overall effect of antennectomy on recovery time. Antennectomy did not reduce recovery time significantly ($F = 0.54$. $d.f = 1,10$ $p > 0.05$). On the basis of individual beetles, there was no trend; some beetles became more sensitive while others became less sensitive after antennectomy (Figure 24).

Blinding

This experiment was designed to determine the role eyes may play in the indirect detection of vibration. Normal beetles (i.e. eyes not painted), were startled with a frequency of 30 Hz and a displacement of 1.3 mm. After startling, beetles were blinded by painting their eyes with pen touch quick-dry silver metallic marker. They were given 24h to recover. Beetles were startled again after the 16 hour period. In both states each beetle was startled 3 times and the mean recovery time was obtained for each beetle.

Figure 25 shows the effect of blinding on recovery time. Blinding did not reduce recovery time significantly ($F = 1.40$. d.f. $= 1,20$ $p>0.05$). On the basis of individual beetles there was no trend; some beetles became more sensitive while others became less sensitive after blinding (Figure 26).

Histology

The pro-, meso- and metathoracic legs of newly emerged adult male and female potato beetles with soft cuticle were used for these observations. Legs were either removed in their entirety or sectioned proximal to the coxae and distally below the femur-tibial joint. The purpose of using whole legs was to find out if the tarsomeres contained any chordotonal organs. The legs were fixed in Bouin's fixative for 24h. After fixation, they were dehydrated in a graded ethanol series and embedded in histological paraplast. Longitudinal and transverse sections, 5-10μm thick were cut on a 2030 Biocut microtome. These sections were stained with Hubschman's Azan stain (Hubschman, 1962) and mounted in D.P.X neutral mounting medium. Sections were subsequently photographed with Polyvar photographic equipment.

I found a chordotonal organ (co) in the trochantero-femoral articulation of all three legs, closely associated with the trachea (tr) (Figure 27). In addition, a novel

organ is present only in the prothoracic leg. This organ is located proximal to the femoro-tibial articulation of the foreleg and it is closely associated with the main trachea of the leg. It contains only three scolopidia. The organ is spindle-shaped and it is attached to the cuticle (Figure 28). No chordotonal organs were found in the tibio-tarsal joints.

Foreleg Amputation

Since the novel organ was found only in the forelegs, the following experiments were designed to test if the forelegs were solely responsible for the detection of vibration. Two experimental protocols were employed.

Protocol 1

First, 8 normal beetles were startled with a frequency of 30 Hz and a displacement of 1.3 mm. Subsequently, their forelegs were removed with iris scissors and they were given 48h to recover. They were startled again after the 48h period. Both unoperated and operated beetles were startled 3 times.

Both operated and unoperated beetles were very responsive to the stimuli used. However, the mean recovery time of 231 $\pm$ 65 sec for unoperated beetles dropped to 107 $\pm$ 11 sec after operation, this difference was jut short of significance at the 0.05 level ($F = 3.51$, d.f = 1, 14, $p = 0.08$).

Protocol 2

Since there was a drop in recovery time after amputation, the forelegs might contribute some information relating to the length of recovery. The following experiment examined this question, using a different approach. Two groups of beetles, control and experimental were used. The control group consisted of 4 day-old beetles

of both sexes and they were maintained on fresh leaves until used. The experimental group had their forelegs amputated just below the trochanter-femur articulation with iris scissors, when they were 2 days old. Beetles were then given 2 days on a host plant to recover from the operation, and then tested at the same age as the control group. Each beetle from the two groups was startled three times with a frequency of 30 Hz and a displacement of 1.3 mm. The above experiment was done in two parts; in December 1993 and in January, 1994. A total of 17 beetles was used for the December experiment and 14 for the January experiment.

For both unoperated and operated beetles, the mean recovery time for beetles tested in January was more variable than for those tested in December. For the unoperated groups, the mean recovery time for beetles tested in December was not significantly different from that for beetles tested in January i.e. 225 $\pm$ 35 vs. 476 $\pm$ 141 sec {Prob ($|Z| > 0.078 = 0.9376$} while for the operated groups the mean recovery times for beetles tested in both months were different i.e. 142 $\pm$ 36 vs. 317 $\pm$ 47 sec {Prob ($|Z| > 2.966 = 0.003$}. Data could therefore not be pooled, so the December and January experiments were analyzed separately. Both operated and unoperated beetles were very responsive to the stimuli used. With foreleg amputation, there was a decrease in recovery time from 225 $\pm$ 35 to 142 $\pm$ 36 ($F = 2.66$, d.f = 1,43, $p = 0.1$) sec and from 476 $\pm$ 141 to 317 $\pm$ 47 ($F = 1.15$, d.f = 1,40, $p = 0.3$) sec for December and January experiments respectively, these differences were not significant.

Discussion

The antennae and eyes are not involved in vibration detection since some beetles showed decreased recovery times while others showed increased recovery times for both the antennectomy and blinding experiments. Chordotonal organs found in the

trochantero-femoral joint of all three legs as well as the novel organ found in the femoro-tibial may play a role in vibration detection. The novel organ could not be classified as a subgenual organ because it lies at the distal end of the tibia, i.e. it is proximal to the femur-tibial joint, and subgenual organs are usually located just below the femur-tibia joint (Bullock and Horridge, 1965).

The drop in recovery time with foreleg amputation suggests that the novel organ might provide some information relating to the length of recovery. Though ants have subgenual organs, they detect vibratory stimuli by means of three groups of companiform sensilla placed between the trochanter and femur (Markl, 1970). It is possible that the chordotonal organs found between the trochantero-femoral joint of all three legs are involved in the detection of vibration in this beetle. Also in the honey bee, *Apis mellifera,* all three legs are involved in vibration detection (Little, 1962).

The nervous system might mediate the startle response because in *Ranatra*, removal of the supraesophageal ganglion causes a marked reduction in the duration of thanatosis (Holmes, 1906). Though speculative, it is possible that biogenic amines or neuroactive peptides are involved in regulating the length of recovery period. In insects, biogenic amines regulate behavioral responsiveness such as sensitization and arousal (Evans, 1980). Octopamine is known to cause general or increased responsiveness (Kinnamon et. al., 1984; Flamm and Harris-Warrick, 1986; Sombati and Hoyle, 1984a and O'Shea and Evans, 1979) while serotonin causes an opposite effect (Glanzman and Krasne, 1983; Harris-Warrick and Kravitz, 1984). Also the concentration of octopamine in insect haemolymph varies with the degree of arousal of the insect. Levels increase in response to a variety of stressful stimuli (Orchard et. al., 1981; Davenport and Evans, 1984b). In the American cockroach, *Periplaneta americana* the haemolymph titre of octopamine is elevated by handling or mechanical stress (Bailey et.al., 1983).

In the Colorado potato beetle, it is possible that octopamine and serotonin might play a role in the startle response with the rate of release of these chemicals depending upon the prevailing conditions during startle. For example with repeated startles, where there is a trend of sensitization followed by habituation, either the rate of release of serotonin increases initially and then declines with repeated startles or octopamine is released at an increasing rate after a number of startles resulting in habituation. Also startle within the immobile phase prolonged recovery time. Under this startle regime it is possible that serotonin is released with each additional startle or the release of octopamine into the nervous system is suppressed. Alternatively, it is possible that under this regime octopamine is metabolised at a faster rate.

In *Schistocerca gregaria*, starvation led to increased titres of octopamine in the haemolymph (Davenport and Evans, 1984). In starved beetles where recovery times were shorter compared to fed beetles, there might be an increase in the rate of release of octopamine thus making beetles more active. There is also the possibility that an unknown neuromodulator might be involved in the regulation of the startle response in the Colorado potato beetle.

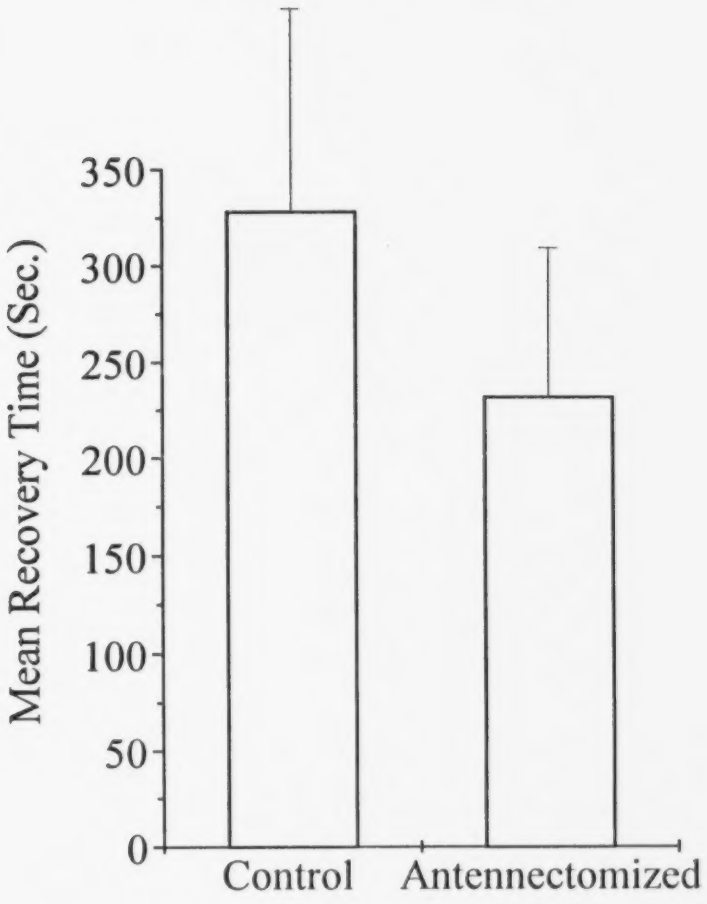

Figure 23. Effect of antennectomy on recovery time. The same group of beetles were used for both the control and antennectomy. The recovery time for the antennectomized group was not significantly different from that of the control group ($F = 0.54$. d.f = 1,10 $p > 0.05$). N = 6.

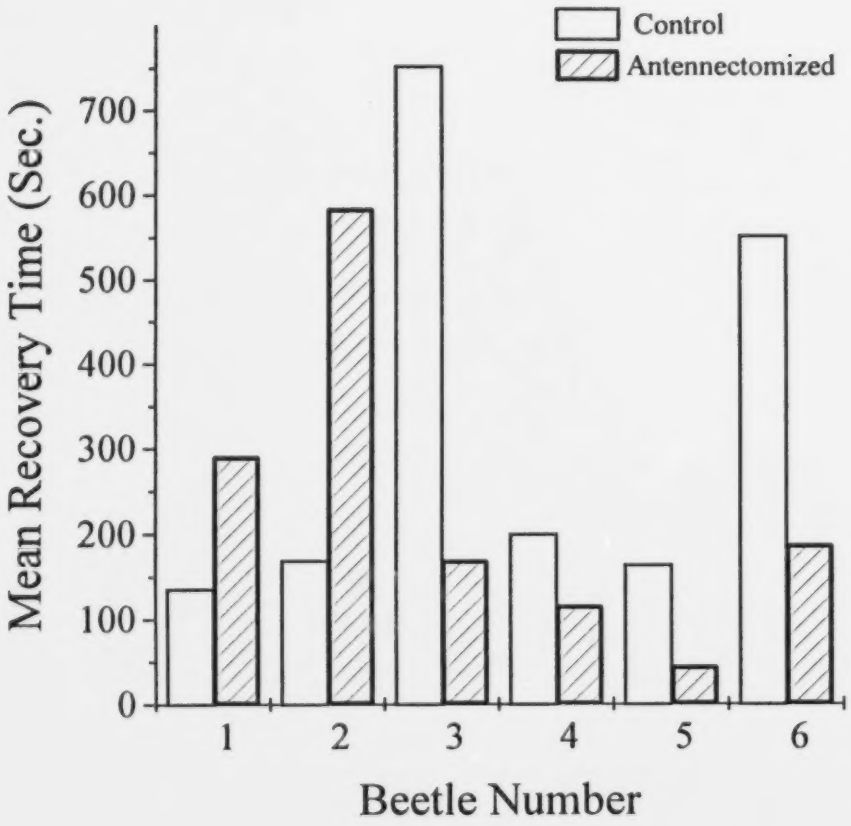

Figure 24. Graph showing the effect of antennectomy on individual recovery times. No trend was observed in individual beetles.

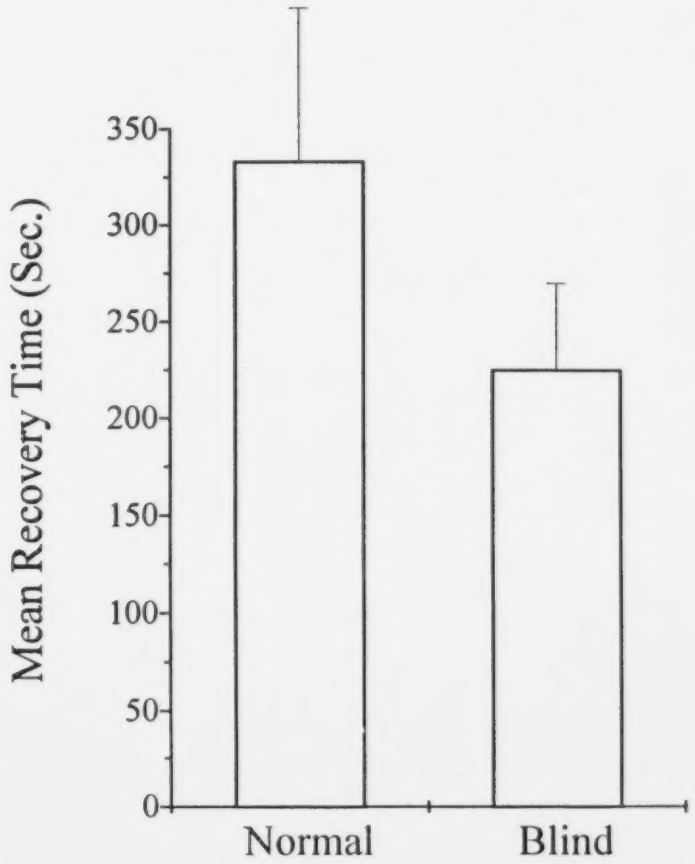

Figure 25. Effect of sight on recovery time. The same group of beetles were used for both the control and blinding experiments. The recovery time for the blind group was not significantly different from that of the control group ($F = 1.40$ d.f = 1,20 $p>0.05$). N = 11.

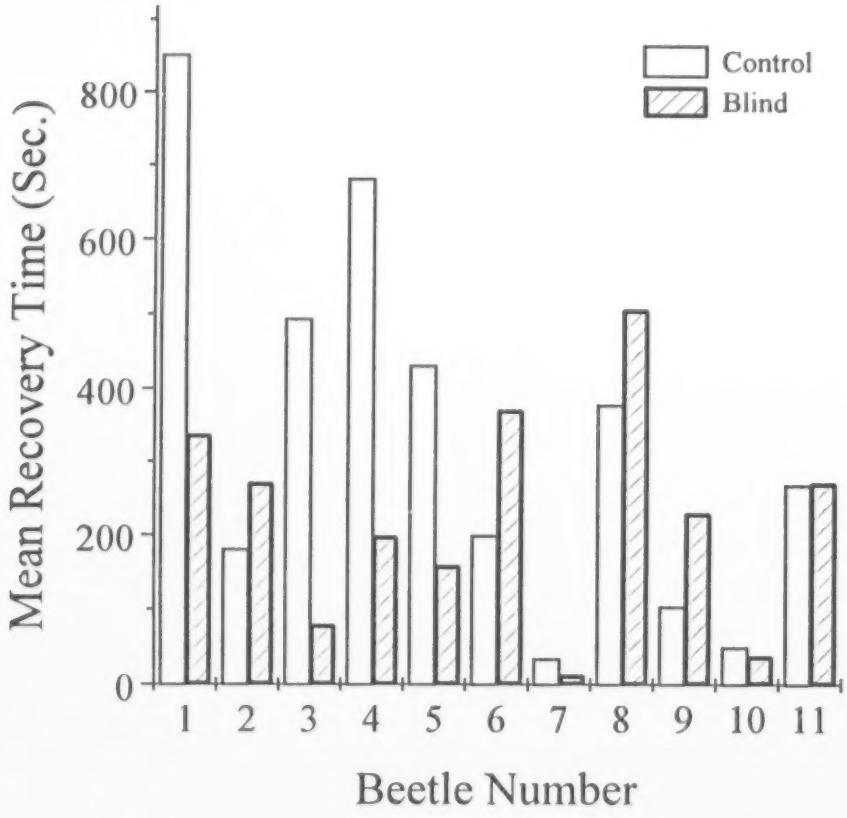

Figure 26. Graph showing the effect of blinding on individual recovery times. No trend was observed in individual beetles.

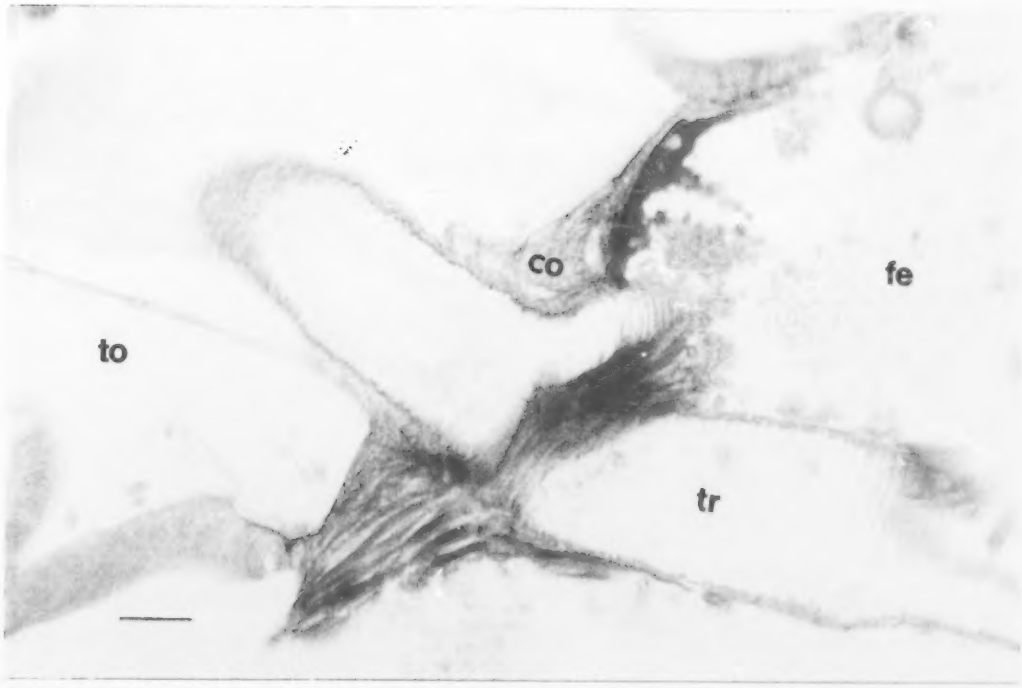

Figure 27. Longitudinal section of a chordotonal organ (co) between the trochantero-femoral joint in the foreleg of the Colorado potato beetle. Thickness = 7μm. Scale line represents 56 μm. co = chordotonal organ, fe = femur, to = trochanter and tr = trachea.

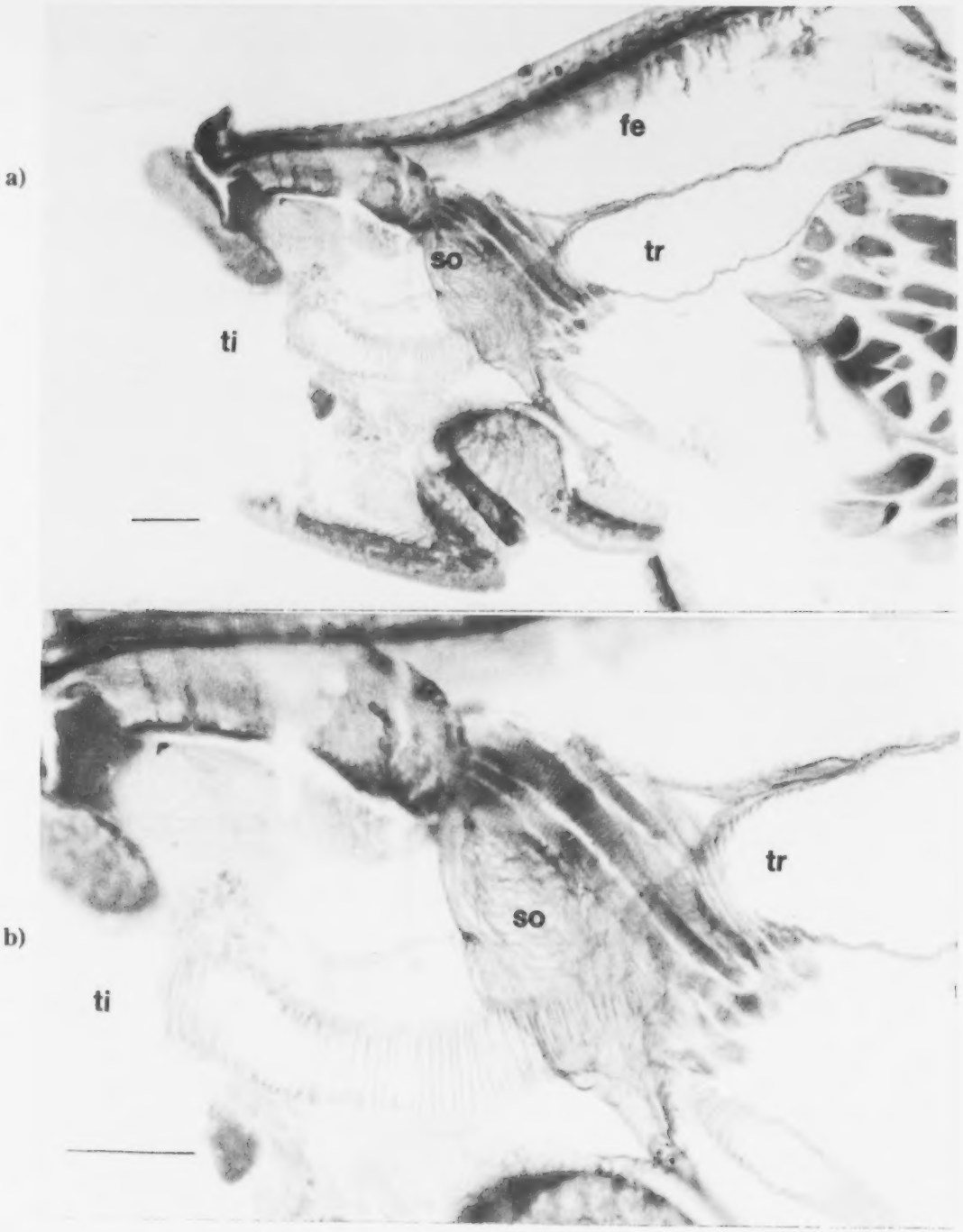

Figure 28 a & b. Longitudinal section of a novel organ in the foreleg of the Colorado potato beetle. Thickness = 7μm. Scale lines represent 56 μm. fe = femur, so = novel organ, ti = tibia and tr = trachea.

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